Soil enzymes are preferentially associated with larger particles in highly organic Arctic tundra soils

Jane Martinez1,*, Jennie McLaren1, Craig E. Tweedie1, and Anthony Darrouzet-Nardi1

Microbial processes, including extracellular enzyme (exoenzyme) production, are a major driver of decomposition and a current topic of interest in Arctic soils due to the effects of climate warming. While enzyme activity levels are often assessed, we lack information on the specific location of these exoenzymes within the soil matrix. Identifying the locations of different soil enzymes is needed to improve our understanding of microbial and overall ecosystem function. Using soil obtained from Utqiaġvik, Alaska, our objectives in the study are (1) to measure the activity of enzymes in soil pore water, (2) to examine the distribution of activity among soil particle size fractions using filtration, and (3) to cross these particle size fraction analyses with disruption techniques (blending to shred and sonication to further separate clumped/aggregated soil materials) to assess how tightly bound the enzymes are to the particles. The results of the soil pore water assays showed little to no enzyme activity (<0.05 nmol g soil⁻¹ h⁻¹), suggesting that enzymes are not abundant in soil pore water. In the soil cores, we detected activity for most of the hydrolytic enzymes, and there were clear differences among the particle size and disruption treatments. Higher activities in unfiltered and 50-μm filters relative to much finer 2-μm filters suggested that the enzymes were preferentially associated with larger particles in the soil, likely the organic material that makes up the bulk of these Arctic soils. Furthermore, in the sonication + blending treatment with no filter, 5 of 6 hydrolytic enzymes showed higher activity compared to blending only (and much higher than sonication only), further indicating that enzyme–substrate complexes throughout the organic matter component of the soil matrix are the sites of hydrolytic enzyme activity. These results suggest that the enzymes are likely bound to either the producing microbes, which are bound to the substrates, or directly to the larger organic substrates they are decomposing. This close-proximity binding may potentially minimize the transport of decomposition products away from the microbes that produce them.

Keywords: Extracellular enzymes, Soil enzyme activity, Arctic soils, Decomposition, Exoenzymes

Introduction
Decomposition of organic matter in soils is driven by microbial biochemical activity, specifically by producing hydrolytic and oxidative enzymes (Skujins and Burns, 1976; Burns, 1982). Microbes produce soil enzymes to access nutrients required for metabolic processes and growth (Allison and Vitousek, 2005; Dick et al., 2011). Despite the importance and ubiquity of this process, the location of soil enzyme activity within the soil matrix is incompletely known. There are many distinct locations within the soil matrix where these enzymes might operate, such as free-floating in soil pore water, attached to the microbial cells, linked with enzyme–substrate complexes, associated with soil organic matter (SOM; Burns, 1982), or associated with clay–enzyme or tannin–enzyme complexes (Burns, 1986; Marx et al., 2005). These different locations vary in their mobility within the soil and their proximity to the enzyme-producing microbes. Identifying the location of soil enzymes is needed to improve our basic understanding of soil microbial functioning, helping to illuminate how microbes interact with their substrates (Burns et al., 2013) and how they guarantee a return on investment for the microbes that create them (Treseder and Vitousek, 2001). In addition, less work has been done on assessing enzyme activity in soil pore water than on potential association with soil particles, yet it is an important exchange depot for soil materials and thus worth quantifying.

Arctic soils are an especially relevant system to study the location of enzyme activities because of the possibility for accelerated decomposition due to climate change (Schuur et al., 2015). These soils remain frozen for most of the year, and the low temperatures preserve decomposed material in the surface layers (Weintraub and Schimel, 2003; Walz et al., 2017). The accumulation of large stores of decomposed material has created a thick layer of organic soils, with tundra soils containing approximately 50% of the world’s soil carbon (Shaver et al., 2006; Tarnocai, 2009). Due to temperature increases, large soil carbon
reserves in the permafrost are vulnerable to decomposition, the increase of nutrient availability, and the release of CO₂ to the atmosphere (Nadelhoffer et al., 1991; Schuur et al., 2013). With these climate change effects, gaining better knowledge of enzyme activity locations within the soil matrix will help to enable a better mechanistic understanding of how enzymes will respond to increasing temperatures in cases such as deeper active layers, longer growing seasons, and changing moisture conditions in the soil.

In Arctic soils, enzymes could be associated with various soil matrix components such as soil pore water, SOM, higher molecular weight compounds such as tannins, or microbial cells, (Wallenstein and Burns, 2015). Investigations of enzyme locations often point to particle size as a determinant of enzyme location. For example, substantial differences between particle sizes were observed in a grassland ecosystem depending on enzyme identity (Marx et al., 2005). Also, a fertilization experiment on crop soils determined that particle sizes 63–200 μm had the highest enzyme activities (Zhang et al., 2015). However, these more mineral soils differ substantially from Arctic soils, which are highly organic and frequently water-saturated. Besides particle size, aggregation of soil materials can also affect enzyme location, given that it can physically prevent enzymes from being exposed, thus limiting our ability to measure activity. For example, disaggregation by disruption of soils by grinding has for example shown an increase in the measurable activity of both protease and β-glucosaminidase, suggesting that the enzymes were inside the aggregates (Fukumasu and Shaw, 2017). Moreover, the disruption of soils can release enzymes and microorganisms entrapped in clustered soil particulates by sonication (Kaiser and Asefaw Berhe, 2014). Another factor to consider is that separating the soil particles by performing physical disturbances, such as microwaves, waterbath, blending, ultrasonic and laser treatment to soils, may lead to cell lysis (McMillan et al., 2013). This cellular damage can expose intracellular or cell-bound enzymes (Wallenstein and Burns, 2015). Because of the substantially different nature of the soil matrix in organic horizons of Arctic soil, further investigations of enzyme locations are warranted.

This study aims to explore the possible location of Arctic soil enzymes within the soil matrix using various separation techniques. Our objectives in the study are (1) to measure the activity of enzymes free-floating in the soil pore water, (2) to examine the effect of the distribution of activity among soil particle size fractions using filtration, and (3) to cross these particle size fraction analyses with disruption techniques within the soil matrix (blending to shred and sonication to separate further clumped/aggregated soil materials) to assess how tightly bound the enzymes are to the particles. These investigations of particle size crossed with varying levels of disruption will help to narrow down where in the soil matrix the greatest soil potential enzyme activity is occurring. We hypothesize that the soil enzymes are associated with the large organic matter substrates, and thus, fractions containing these substrates will have the highest potential activities. To examine this hypothesis, we assayed free-floating enzyme activities in soil pore water samples and assessed hydrolytic and oxidative enzyme activities from soil cores using blending, filtration, and sonication techniques separately and in combination.

Materials and methods

Research site

Soil cores were collected in Summer 2016 from field locations near Utqiaġvik, Alaska, located on the northern coast and Atqasuk (70.47778°N, 157.41806°W), a village located 60 miles southwest of Utqiaġvik (Figure 1). The mean annual air temperature is –12°C (Meisel et al.,
2021). During the summer months, June–August, the temperature can rise to 4°C (Gädeke et al., 2021). The climate from the last 30 years (1991–2020) is reported by the National Oceanic and Atmospheric Administration (NOAA’s National Centers for Environmental Information, 2020): mean annual temperature is −10.7°C, and mean annual precipitation is 15.7 cm. Additionally, recent years have shown that the soil temperatures have been increasing over time (Hollister et al., 2006; May et al., 2020). Historical data show that the changes in mean annual air temperature are controlled by the winter months. From 1921 to 1995, the long-term means were 3.3°C during the winter and 1.6°C in the summer months (Zhang and Stamnes, 1998). The general mean soil temperature can vary in the summer months from 3.6°C to 5°C (Hinkel et al., 2001). This ecosystem is dominated by ice-wedge polygonal tundra spanning drained thaw-lake basins and interstitial tundra (Silikowska et al., 2019). Vegetation at this site consists of moist acidic tundra plant communities, including lichens, bryophytes, graminoids, and shrubs, which fluctuate in vegetation cover with herbivory outbreaks (Haugen and Brown, 1980; Lara et al., 2015). Additionally, it has been shown that there was continuous permafrost at Utqiagvik from 1950 through the late 1970s, classifying the soils as Gelisols (Nachtergaele, 2001). More recently, soils have generally warmed (Villarreal et al., 2012), thus making it relevant to study decomposition processes in this area. In Utqiagvik, the young (<10 cm deep) and medium-aged (10–15 cm) basins contain an organic layer, and soils are classified as Aquorthels and Aquiturbels (Soil Survey Staff, 1999; Hinkel et al., 2003). In this region, the active layer, which is the maximum thaw depth by the end of the summer (Schafer et al., 2011), is between 40–60 cm deep (Clayton et al., 2021). In the Utqiagvik Peninsula, it has been shown that roughly 100.3 mg/g can be classified as clay material (<2 μm size) from a particle size fractionation of an Aquiturbel soil (Mueller et al., 2017). For the purposes of this study, we sampled across a wide geographic area to represent the spatial variation in soils across the North Slope shown in Table 1. We designed this study with the expectation that the loci of enzyme activities in the soil are relatively general across space within these Arctic systems. Thus, while this widely dispersed geographic design risks the variation being high, if we can demonstrate effects, this would be evidence that the documented trends are more general.

| Soil Sample | Collection Site | Location on Collection Site | Date | Tundra Class | Vegetation | Latitude–Longitude |
|-------------|-----------------|-----------------------------|------|--------------|------------|-------------------|
| S1          | Utqiagvik, AK   | ITEX/BEO                    | July 21, 2016 | Dry-moist dwarf shrub-graminoid tundra | Sphagnum spp. | 71.31371–156.58887 |
| S2          | Utqiagvik, AK   | Gaswell Rd                  | August 13, 2016 | Moist graminoid tundra | Eriophorum scheuchzeri | 71.25814–156.33311 |
| S3          | Utqiagvik, AK   | BEO                         | July 16, 2016  | Moist graminoid tundra | E. scheuchzeri | 71.31403–156.59401 |
| S4          | Utqiagvik, AK   | Gaswell Rd                  | August 8, 2016 | Moist graminoid tundra | E. scheuchzeri | 71.28325–156.43034 |
| S5          | Utqiagvik, AK   | Snow Fence/BarC             | July 14, 2016  | Moist graminoid tundra | E. scheuchzeri | 71.31303–156.592662 |
| S6          | Utqiagvik, AK   | Hospital Vehicle Tracks     | July 30, 2016  | Wet graminoid tundra | E. scheuchzeri | 71.29814–156.7361 |
| S7          | Utqiagvik, AK   | Hospital Vehicle Tracks     | July 30, 2016  | Dry dwarf shrub-graminoid tundra | E. angustifolium | 71.29793–156.73387 |
| S8          | Utqiagvik, AK   | Cake Eater Rd               | July 20, 2016  | Successional-dry dwarf shrub-graminoid tundra | Salix rotundifolia | 71.25736–156.53288 |
| S9          | Utqiagvik, AK   | Vehicle Tracks BARC         | July 11, 2016  | Successional-dry dwarf shrub-graminoid tundra | Potentilla hypartica | 71.32113–156.67395 |
| S10         | Atqasuk, AK     | MISP GRID                   | August 6, 2016 | Successional-dry dwarf shrub-graminoid tundra | Papaver hultenii | 70.501957–157.434615 |

Areas used to identify the soil collected: BEO = The Barrow Environmental Observatory; BARC = Barrow Arctic Research Facility; MISP = Mobile Instrumented Sensor Platforms; ITEX = International Tundra Experiment.
across different soil locations and types. The soil core samples were 5 cm in diameter and 15 cm in depth, made from cylindrical metal and hammered with a rubber mallet into the soil. To have a wide range of site representation of disturbed and undisturbed areas, we collected from inland moist acidic tundra regions and close to the coast (approximately 2–5 m away), snow fences, and within vehicle track disturbances (Table 1).

**Soil pore water assays**

To examine free-floating enzymes in the soil pore water, we used rhizon samplers (Eijkelkamp North America Inc., Morrisville, NC, USA, Part No. 192101) for soil pore water collection. These allow access to the continuous water within the soil matrix but not necessarily water-bound at low matric potential (Saiya-Cork et al., 2002; Dick et al., 2011; Darrouzet-Nardi and Weintraub, 2014). The rhizon samplers consist of 4 main parts: a hydrophilic porous polymer, stainless steel wire, polyvinylchloride tube, and the lock connector. We attached a needle to the lock connector, and soil pore water was acquired using a 6-ml vacuette (Greiner Vacuette No. 456089; Darrouzet-Nardi and Weintraub, 2014). Rhizon sampler samples were collected near the plots from the International Tundra Experiment, site located in Barrow, Alaska (samples shown in Supplemental Material Table S1). The tubes were attached to the needles and collected within 24 h. Once the sample was recovered, it was placed with the soil samples in the freezer at –30°C. From these samples, fluorometric measurements were conducted as described above to test the potential enzyme activity (Henry and Molau, 1997). In addition, to compare with the potential enzyme activities measured in the soil core extractions, we performed a dimensional analysis based on the amount of soil mass represented by the measured soil water volume in both cases (Supplemental Material S1).

**Separation treatments**

We applied 8 different treatments (Table 2) to separate subsamples for each of the 10 samples collected using our standard protocol (blending with no filter). To test if enzymes were associated with SOM or with other particles, after blending, we then applied 2 separation techniques factorially: sonication (with and without sonication) and filtering (no filter, 50 μm, and 2 μm). The filters separate the soil by particle size fractions. To assess the total soil matrix including all organic matter particles and its constituents, no filter was used. The 50-μm filter removed larger (primarily organic) particles. Consequently, to separate most particles sized 2–50 μm from particles sized <2 μm (German et al., 2011), a 2-μm filter was used. Similar soils from this Arctic region have been analyzed by physical soil fractionation, where it was determined that there is a high amount of particulate organic matter not associated with soil minerals (Mueller et al., 2017).

Alongside particle size fractionation, sonication was used as a physical disruption technique to clumped and aggregated soil from itself and possibly separate enzymes or cells from soil materials. Sonication is generally less harsh and does not shred soil particles as blending does. Sonication generates pressure waves and can disperse soil aggregates at low energy applications without damaging the microbes or causing cell lysis (Amelung and Zech, 1999; Kaiser et al., 2012).

### Table 2. Separation treatments and soil pore water. DOI: https://doi.org/10.1525/elementa.2021.00020.t2

| Identifier | Treatment | Purpose of Treatment | Blend | Sonicate | Filter |
|------------|-----------|----------------------|-------|----------|--------|
| Control    | Blend     | Shred soil organic particles and compare disruption to sonication | Yes   | No       | No filter |
| Treatment 1| Blend + Filter 50 μm | Remove larger particles, mostly organic particles | Yes   | No       | 50 μm   |
| Treatment 2| Blend + Filter 2 μm | To separate silt-sized particles (2–50 μm) from clay-sized particles (<2 μm) | Yes   | No       | 2 μm    |
| Treatment 3| Sonicated (Son.) | Separate organic particles | No    | Yes      | No filter |
| Treatment 4| Son. + Filter 50 μm | Compare with Treatments 1 and 7 | No    | Yes      | 50 μm   |
| Treatment 5| Son. + Filter 2 μm | Compare with Treatments 2 and 8 | No    | Yes      | 2 μm    |
| Treatment 6| Son. + Blend | Potentially extract the enzymes and bacteria from bulk soil or soil fractions | Yes   | Yes      | No filter |
| Treatment 7| Son. + Blend + Filter 50 μm | Same as Treatment 6 + removed larger particles mostly organic particles (larger than 50 μm) | Yes   | Yes      | 50 μm   |
| Treatment 8| Son. + Blend + Filter 2 μm | Same as Treatment 6 + to separate silt-sized particles (2–50 μm) from clay-sized particles (<2 μm) | Yes   | Yes      | 2 μm    |
| Soil pore water | Lysimetry | Determine whether enzymes are free-floating in the soil matrix | No    | No       | No filter |
Treatment 1 (blend + 50-μm filter) and Treatment 2 (blend + 2-μm filter) were run through the treatment’s respective filter after the soil was blended. The filtration was to distinguish the particle size at which enzyme activities could be detected within the soil matrix and possibly determine location by removing larger particles (reference Figure 2 for visual details). Treatments 3, 4, and 5 (no blend + sonicate, no blend + sonicate + 50-μm filter, no blend + sonicate + 2-μm filter) did not get shredded by the blender. These treatments were sonicated with a VWR Ultrasonic bath (VWR Ultrasonic Cleaner, Radnor, PA) at a low amplitude of 20–30 kHz for 5 min per sample. Sonication was at a low setting to avoid cell lysis but allow for dispersion of soil aggregates (Hunter and Busacca, 1989; Kaiser et al., 2012). After the sonication process took place, Treatment 3 was transferred to the 96-well plate for fluorometric analysis.

**Soil potential enzyme activity assays**

We used a fluorometric soil activity assay protocol to quantify soil decomposition enzyme activity within the soil matrix (Romanovsky and Osterkamp, 2000). Enzyme assays were performed with a high throughput microplate reader technique. We analyzed the samples from the activity of 9 enzymes: 2 oxidative and 7 hydrolytic (Table 3). It is important to note that the enzyme activity we measure...
is potential enzyme activity in the laboratory and not in situ. In situ activity refers to enzymes under natural conditions, as opposed to potential enzyme activity, which measures the maximum reaction rate of an enzyme-catalyzed reaction at a specific temperature with no substrate limitation (Wallenstein and Weintraub, 2008). Soil slurry samples were prepared by mixing 1 g soil and 125 ml of 50 mM sodium acetate buffer (pH 5). The samples were kept stirring with a magnetic plate until transferred to the black (hydrolytic) and translucent (oxidative) 96 microwell plates. There were 2 types of fluorescent standards for the hydrolytic enzymes: 7-amino-4-methylcoumarin (MC) and 4-methylumbelliferyl (MUB). The MC standards were used for leucine-amino-peptidase and MUB for the other enzymes (see Table 3).

Control quench samples were made (soil slurry and MUB/MC) to correct MUB/MC fluorescence intensity due to soil turbidity (Marx et al., 2001). Additionally, measurements were taken of the background fluorescence of soils, substrates, and MUB/MC standard curves (Marx et al., 2001; Saiya-Cork et al., 2002; Sinsabaugh et al., 2003; McLaren et al., 2017). The microplate reader read the black plates to check the fluorescent activity, and the oxidative plates were incubated for 24 h, then run in a BioTek Synergy HT microplate reader (BioTek Instruments Inc., Winooski, VT, USA; German et al., 2011).

**Results**

**Soil pore water enzyme assay**

Activities of 4 of the 6 enzymes were detectable from the assays conducted on soil water from the rhizon sampler technique used to collect soil pore water (Figure 3). N-acetyl-glucosaminidase and xylosidase had extremely small means, close to nondetectable. We observed measurable activity in α-glucosidase, β-glucosidase, cellobiohydrolase, and phosphatase. However, after adjusting for the mass of soil represented, activities were very low compared to the soil core samples, with averages less than 0.05 nmol g⁻¹ soil h⁻¹ (Figure 3; Supplemental Material S1). Thus, there was no significant difference between the enzymes.

**Soil cores—Hydrolytic enzymes**

In the soil core samples, measurable activity was present for most hydrolytic enzymes assayed with blending and the various separation techniques. In most cases, the activity dropped when a filter was added to a treatment. It stayed consistent from highest to lowest according to the corresponding filter size. Although activity was low at 2 μm across all enzymes, activity was still measurable. In α-glucosidase, there is a significant difference between the sonicated and the sonicate + blend treatment, the filters

### Table 3.

**Summary on enzyme functions and substrates.** DOI: https://doi.org/10.1525/elementa.2021.00020.t3

| Hydrolytic Enzymes | Abbreviation | Function | Substrate | Fluorescent Standards |
|--------------------|--------------|----------|-----------|-----------------------|
| β-1,4-Glucosidase  | BGLUC        | Releases glucose from cellulose, optimal activity at 85°C | 4-MUB-β-D-Glucosidase | MUB |
| α-1,4-Glucosidase  | AGLUC        | Releases glucose from soluble saccharides | 4-MUB-α-D-Glucosidase | MUB |
| β-D-1,4-Cellobiohydrolase | CELLO | Releases disaccharides from cellulose | 4-MUB-β-D-Cellobiose | MUB |
| β-1,4-Xylosidase   | XYLO         | Degrades hemi-cellulose | 4-MUB-β-D-Xylosidase | MUB |
| β-1,4-N-Acetyl-glucosaminidase | NAG | Degrades chitin | 4-MUB-N-Acetyl-β-D-glucosaminide | MUB |
| Phosphatase        | PHOS         | Releases phosphate ions from phosphate group | 4-MUB-Phosphate | MUB |
| Leucine-amino-peptidase | LAP | Degrades protein into amino acids | 7-Amino-4-methylcoumarin | MC |
| Oxidative Enzymes  | Abbreviation | Function | Substrate | |
| Phenol oxidase     | PHENOL       | Oxidizes phenols and consume oxygen | L-Dopa (L-3,4 Dihydroxyphenylalanine) | |
| Peroxidase         | PEROX        | Use H₂O₂ as an electron acceptor | L-Dopa (L-3,4 Dihydroxyphenylalanine) | |

MC = 7-amino-4-methylcoumarin; MUB = 4-methylumbelliferyl.
had less of an effect. The means of 5 of the 6 hydrolytic enzymes (all except cellobiohydrolase) at blend + sonicate showed higher enzyme activity. Cellobiohydrolase has its highest activity with just blending, additional treatments did not show higher activity. The activity of most enzymes did not increase with the sonication treatment alone (Figure 4).

**Oxidative enzyme assays**

For phenol oxidase, sonication did not affect the enzyme activity ($p > .05$). Additionally, when blending was performed on phenol oxidase, it showed no activity ($\text{mean} = 0.0$). When sonication is applied, there was a slight average activity of 2.4 nmol g$^{-1}$ soil h$^{-1}$. In peroxidase, sonication had a significant effect compared to the blended treatment ($p < .05$). In both phenol oxidase and peroxidase, when looking at blend + 50-μm filter, the means are higher for both than in blending with filtration of 2 μm ($p < .05$). However, there is no significant difference between most of the sonicated treatments (Figure 5).

**Discussion**

We sought to better understand enzyme location in Arctic tundra soils. To do this, we quantified the enzyme activity of 2 oxidative and 7 hydrolytic enzymes by the distribution of activity among size fractions and disruption of soil aggregates. Our results suggest that most of the enzymes in this region are associated with large particles of SOM and are not highly abundant in soil pore water. Additionally, there were clear differences among the particle size and disruption treatments. In the following sections, we will discuss why enzymes are not free-floating in the soil pore water, why the enzymes are not located in the small size fraction (<2 μm), and how enzymes are likely tightly bound to the organic matter in tundra soils. Overall, we will make the case that the enzymes are likely bound either directly to the decomposition substrate (the large partially decomposed organic materials in the soil matrix) or to microbial cells that are bound to the substrate.

**Soil pore water**

The results of soil pore water assays showed very low potential enzyme activities once corrected for the soil volume represented (<0.05 nmol g$^{-1}$ h$^{-1}$), suggesting that enzymes are not abundant in soil pore water. Microorganisms might secrete the enzymes into the soil water, but enzyme allocation has been shown to shift with changes in dissolved organic carbon and nutrient availability (Allison and Vitousek, 2005; Seifert-Monson et al., 2014). Changes in the soil potentially may explain why they do not remain in the pore water for a long time. Our pore water results show some low but detectable enzyme activity on some enzymes like α-glucosidase and phosphatase, where we may observe the activity of highly transitory enzymes moving toward the substrate. Since α-glucosidase and phosphatase were still low in potential activity, the slight increase does not suggest that these enzymes are unusual with respect to the others tested. Furthermore, a recent study using microdialysis worked to analyze free soil enzymes in situ, it was demonstrated...
that 91% of the total hydrolytic activity were not free-floating enzymes (Buckley et al., 2019), thus, supporting our results and agreeing that the free enzyme pools are rapidly changing. Moreover, in our study, when using soil core samples, typically there are higher enzymatic activities than when using the soil pore water samples, which is why our data suggest that enzymes are physically bound to soil particles (Steinweg et al., 2012; Bell et al., 2013) or cells (Burns, 1982), and are not free-floating in the pore spaces.

**Enzyme activity among particle sizes in the soil**

Our filtration treatment results showed higher activities in unfiltered and 50-μm filters relative to much finer 2-μm filters, suggesting that all the enzymes we measured related to C, N, and P-acquisition were preferentially associated with larger particles in the soil, likely the organic material that makes up the bulk of these Arctic soils. It is worth clarifying that the total mass of the solid-phase components of the soil decreases with each filtration, indicating that the enzyme activity may have maintained

---

**Figure 4. Hydrolytic enzyme potential activity.** Enzyme activities were calculated in units of nmol g⁻¹ soil h⁻¹ and are color-coordinated by treatment. Letters denote significant differences among treatments based on estimated marginal means with Tukey adjusted p values. DOI: https://doi.org/10.1525/elementa.2021.00020.f4
a similar activity per unit of solid material. Nonetheless, the data indicate limited enzyme mobility through the filter, suggesting they are largely bound to the solid components of the soil matrix, and in these organic soils, these are mainly larger particles. The oxidative enzymes related to lignin degradation had a similar association (Sinsabaugh, 2010). This suggests that the microbes are likely attached or located near the organic matter, which could be important for the producing microbes to ensure return on investment for the enzyme production. Our data do not allow us to discern whether the enzymes are attached to substrates or the microbes. However, the data do show that Arctic soils have higher enzyme activity in the large coarse fraction, supporting our hypothesis of a physical connection between enzyme and substrate.

As we expected based on the known textural differences between these Arctic soils and more mineral soils that have been investigated in other studies, we did not see a significant amount of enzyme activity in the <2 μm fraction. All of the hydrolases had higher activities in the coarser fractions (>50 μm). In contrast with Alaskan soils, there is a higher amount of weathering products, such as clays in warm and humid areas (Jonasson et al., 2001; Hobbie et al., 2002). Soils containing higher clay content, such as a tallgrass prairie, demonstrate abundant enzyme activity in all types of particle size fractions (Borden et al., 2010). Therefore, our data suggest a substantial structural difference in where enzyme activity can expect to be found in these highly organic soils as compared to more typical mineral soils.

High molecular weight particles, such as tannins and humic substances, are also included in the <2 μm fraction and are sometimes thought to be associated with enzymes. For example, one of the main challenges in protein
extraction for soil proteomics has been humic substances, inhibiting protein signaling (Chourey et al., 2010; Chourey and Hettich, 2018). Humic substances are substances formed by the secondary synthesis of a reaction, typically the end product of decaying organic matter (Huang and Hardie, 2009). When decomposition occurs, the complexity of organic material increases, and enzymes have fewer sites available for them to attach to (Ladd et al., 1996). This possibly explains why enzymes are not in the smaller fractions. It has been shown that large fractions hold higher microbial abundance and conditions for microorganisms (Zhang et al., 2015), and enzymes tend to stay close to the microbes that produce them. Nevertheless, our study does show that enzyme proteins are not attached to the freely available humic molecules such as dissolved or suspended organic materials, which would be found in the <2 \( \mu \)m fraction. While we cannot dismiss that humic substances are attached to large particles, we can suggest that enzymes are not primarily located in suspended high molecular complexes (<2 \( \mu \)m).

**The role of soil structure in isolating enzyme activity**

We used 2 disruption effects, sonication and blending, to potentially expose enzymes entrapped in the soil matrix (Fukumasu and Shaw, 2017). Sonication and blending together increased the measured enzyme activities of these Arctic soils the most, indicating that enzymes are likely bound inside undecomposed material. Our results suggest that enzymes in Arctic soils require severe disruption, like blending, to break apart the clumped undecomposed soil material to see more activity. Sonication alone did not have the force to separate the particles due to the relatively high structural integrity of the organic material, which is a notable difference with more mineral soils that can be better dispersed by sonication. For example, xylanase increases with low-energy sonication in a clay loam forest soils (De Cesare et al., 2000), and acid phosphatase increases by 156\% (Rojo et al., 1990) in colluvial Rendzina from a grassland ecosystem. In contrast, in our samples, xylanase activity was nearly the same if using blending or sonication but using them together resulted in higher activity. Because sonication was used alone at a low energy level, substrate-bound enzymes may not have been widely dispersed, and this technique may only be releasing a fraction of the enzymes attached to clumped soil materials. The disruption effects we observed are consistent with the hypothesis that enzymes are tightly bound to the relatively undecomposed organic matter or microbial cells that are themselves bound to the organic matter.

Finally, a noteworthy topic to touch on is the interaction between cell walls and enzymes. Some soil enzymes have been suggested to be attached to cell walls of soil microbes (Stemmer et al., 1998). For example, \( \beta \)-glucosidase, when isolated from culture, has been found to be bound to fungal cell walls (Lynd et al., 2002). While it would require further research, it is possible that blending lyses cells and releases these cell-bound enzymes into the slurry, resulting in higher contact and higher measured enzyme activities. Our results showed that using sonication without blending, we still see either equal or slightly less enzyme activity than with blending. Because sonication is a less invasive method than blending, sonication may not lyse cells and release the inside material, including enzymes. When comparing oxidative and hydrolytic enzymes, there is a slight difference when looking at the oxidative enzymes, particularly in peroxidase, in which sonication and blending together do not show the same synergistic increase we observed in the hydrolytic enzymes. This could be because the oxidative enzymes are less stable in the environment (Sinsabaugh, 2010), and they are negatively correlated to hydrolytic enzymes (Sinsabaugh et al., 2005). Our results suggest that further investigation of whether cellular-bound enzymes contribute to activity would be worth pursuing.

**Conclusion**

Our results revealed a clear trend in which enzymes exhibited minimal (but not zero) activity in the soil pore water. Additionally, we can rule out high enzyme activities in small size fractions (<2 \( \mu \)m) that include clays, high molecular weight molecules such as tannins and humic substances, and cellular debris. Using sonication amplified the enzyme activities in conjunction with blending, suggesting that this technique is causing enzyme release otherwise not seen by blending, suggesting that enzymes are heavily interlaced within the organic substrate and hence more exposure and de-clumping leads to higher measured activities. Overall, our results suggest that soil enzymes are mostly associated with larger particle sizes, likely organic matter, and further studies might focus on differentiating and confirming the type of materials in these larger particle sizes (>2 \( \mu \)m).

**Data accessibility statement**

The data associated with this submission will be archived on the NSF Arctic Data Center (articdata.io) and is also provided as an online supplemental file.

**Supplemental files**

The supplemental files for this article can be found as follows:

- **Text S1.** Enzyme calculations: Soil pore water.
- **Table S1.** Description of rhizon samplers.
- **Data summary.** xlsx

**Acknowledgments**

We would like to thank the Systems Ecology Lab (SEL) at UTEP for the field work contributions and access to field sites and thank Daniela Aguirre for helping with preliminary work for this study. We also want to thank Alma Hernandez for laboratory work.

**Funding**

This study was supported and funded by Dr Anthony Darrouzet-Nardi UTEP startup funds and by National Science Foundation grant (Award #1557162). This material is based upon work supported by the National Science Foundation under Grant No. 1836861 and 1504345. Any
opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.

Competing interests
The authors have declared that no competing interests exist.

Author contributions
Contributed to conception and design: ADN, JKM.
Contributed to acquisition of data: ADN, JM, JKM, CET.
Contributed to analysis and interpretation of data: ADN, JKM.
Drafted and/or revised the article: ADN, JM, JKM.
Approved the submitted version for publication: ADN.

References
Allison, SD, Vitousek, PM. 2005. Responses of extracellular enzymes to simple and complex nutrient inputs. Soil Biology and Biochemistry. DOI: http://dx.doi.org/10.1016/j.soilbio.2004.09.014.

Amelung, W, Zech, W. 1999. Minimisation of organic matter disruption during particle-size fractionation of grassland epipeds. Geoderma. DOI: http://dx.doi.org/10.1016/S0016-7061(99)00023-3.

Bell, CW, Fricks, BE, Rocca, JD, Steinweg, JM, McMahon, SK, Wallenstein, MD. 2013. High-throughput fluorometric measurement of potential soil extracellular enzyme activities. Journal of Visualized Experiments. DOI: http://dx.doi.org/10.3791/50961.

Borden, PW, Ping, C-L, McCarthy, PJ, Naidu, S. 2010. Clay mineralogy in Arctic Tundra Gelisols, Northern Alaska. Soil Science Society of America Journal. DOI: http://dx.doi.org/10.2136/sssaj2009.0187.

Buckley, S, Allen, D, Brackin, R, Jämtgård, S, Nåsholm, T, Schmidt, S. 2019. Microdialysis as an in situ technique for sampling soil enzymes. Soil Biology and Biochemistry 135. DOI: http://dx.doi.org/10.1016/j.soilbio.2019.04.007.

Burns, RG. 1982. Enzyme activity in soil: Location and a possible role in microbial ecology. Soil Biology and Biochemistry 14: 423–427. DOI: http://dx.doi.org/10.1016/0038-0717(82)90099-2.

Burns, RG. 1986. Interaction of enzymes with soil mineral and organic colloids. Interactions of Soil Minerals with Natural Organics and Microbes 17: 429–451.

Burns, RG, DeForest, JL, Marxsen, J, Sinsabaugh, RL, Stromberger, ME, Wallenstein, MD, Weintraub, MN, Zoppini, A. 2013. Soil enzymes in a changing environment: Current knowledge and future directions. Soil Biology and Biochemistry 58: 216–234. DOI: http://dx.doi.org/10.1016/j.soilbio.2012.11.009.

Chourey, K, Hettich, RL. 2018. Utilization of a detergent-based method for direct microbial cellular lysis/proteome extraction from soil samples for metaproteomics studies, in Becher, D ed., Microbial proteomics: Methods and protocols. New York, NY: Springer: 293–302. DOI: http://dx.doi.org/10.1007/978-1-4939-8695-8_20.

Chourey, K, Jansson, J, VerBerkmoes, N, Shah, M, Chavarria, KL, Tom, LM, Brodie, EL, Hettich, RL. 2010. Direct cellular lysis/protein extraction protocol for soil metaproteomics. Journal of Proteome Research 9: 6615–6622. DOI: http://dx.doi.org/10.1021/pr100787q.

Clayton, LK, Schaefer, K, Battaglia, MJ, Bourgeau-Chavez, L, Chen, J, Chen, RH, Chen, AC, Bakiand Dogah, K, Grelik, S, Jafarova, E. 2021. Active layer thickness as a function of soil water content. Environmental Research Letters 16: 5.

Darrouzet-Nardi, A, Weintraub, MN. 2014. Evidence for spatially inaccessible labile N from a comparison of soil core extractions and soil pore water lysimetry. Soil Biology and Biochemistry. DOI: http://dx.doi.org/10.1016/j.soilbio.2014.02.010.

De Cesare, F, Garzillo, AMV, Buonocore, V, Badalucco, L. 2000. Use of sonication for measuring acid phosphatase activity in soil. Soil Biology and Biochemistry. DOI: http://dx.doi.org/10.1016/S0038-0717(99)00212-6.

Dick, RP, Burns, RG, Dick, RP. 2011. A brief history of soil enzymology research. Methods of Soil Enzymology 9. DOI: http://dx.doi.org/10.2136/ssasbookser9.c1.

Fukumasu, J, Shaw, LJ. 2017. The role of macro-aggregation in regulating enzymatic depolymerization of soil organic nitrogen. Soil Biology and Biochemistry 115. DOI: http://dx.doi.org/10.1016/j.soilbio.2017.08.008.

Gädeke, A, Langer, M, Boike, J, Burke, EJ, Chang, J, Head, M, Reyers, CPO, Schaphoff, S, Thierry, W, Thonincke, K. 2021. Climate change reduces winter overland travel across the Pan-Arctic even under low-end global warming scenarios. Environmental Research Letters. DOI: http://dx.doi.org/10.1088/1748-9326/abdcf2.

German, DP, Weintraub, MN, Grandy, AS, Lauber, CL, Rinkes, ZL, Allison, SD. 2011. Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. Soil Biology and Biochemistry. DOI: http://dx.doi.org/10.1016/j.soilbio.2011.03.017.

Haugen, RK, Brown, J. 1980. Coastal-inland distributions of summer air temperature and precipitation in northern Alaska. Arctic, Antarctic, and Alpine Research. DOI: http://dx.doi.org/10.2307/1550491.

Henry, GHR, Molau, U. 1997. Tundra plants and climate change: The International Tundra Experiment (ITEX). Global Change Biology. DOI: http://dx.doi.org/10.1111/j.1365-2486.1997.tb0132.x.

Hinkel, KM, Eissner, WR, Bockheim, JG, Nelson, FE, Peterson, KM, Dai, X. 2003. Spatial extent, age, and carbon stocks in drained Thaw Lake Basins on the barrow Peninsula, Alaska. Arctic, Antarctic, and Alpine Research 35. DOI: http://dx.doi.org/10.1657/1523-0430(2003)035[0291:SEAACS]2.0.CO;2.

Hinkel, KM, Paetzold, F, Nelson, FE, Bockheim, JG. 2001. Patterns of soil temperature and moisture in...
the active layer and upper permafrost at Barrow, Alaska: 1993–1999, in Global and planetary change. DOI: http://dx.doi.org/10.1016/S0921-8181(01)0096-0.

Hobbie, SE, Nadelhoffer, KJ, Högb erg, P. 2002. A synthesis: The role of nutrients as constraints on carbon balances in boreal and Arctic regions, in Plant and soil. DOI: http://dx.doi.org/10.1023/A:1019670731128.

Hollister, RD, Webber, PJ, Nelson, FE, Tweedie, CE. 2006. Soil thaw and temperature response to air warming varies by plant community: Results from an open-top chamber experiment in northern Alaska. Arctic, Antarctic, and Alpine Research 38. DOI: http://dx.doi.org/10.1657/1523-0430(2006)38[206: STATRT]2.0.CO;2.

Hothorn, T, Bretz, F, Westfall, P. 2008. Simultaneous inference in general parametric models. Biometrical Journal. DOI: http://dx.doi.org/10.1002/bimj.200810425.

Huang, PM, Hardie, AG. 2009. Formation mechanisms of humic substances in the environment, in Biophysico-chemical processes involving nonliving organic matter in environmental systems. DOI: http://dx.doi.org/10.1002/9780470494950.ch2.

Hunter, CR, Busacca, AJ. 1989. Dispersion of three andic soils by ultrasonic vibration. Soil Science Society of America Journal. DOI: http://dx.doi.org/10.1002/ssaj.19890361595005300040052x.

Jonasson, S, Chapin, FS, Shaver, GR. 2001. 1.10—Biogeochemistry in the Arctic: Patterns, processes, and controls, in Global biogeochemical cycles in the climate system. DOI: http://dx.doi.org/10.1016/S0161-0526(00)00003-2.

Kaiser, M, Asefaw Berhe, A. 2014. How does sonication affect the mineral and organic constituents of soil aggregates?—A review. Journal of Plant Nutrition and Soil Science. DOI: http://dx.doi.org/10.1002/jpln.201300339.

Kaiser, M, Berhe, AA, Sommer, M, Kleber, M. 2012. Application of ultrasound to disperse soil aggregates of high mechanical stability. Journal of Plant Nutrition and Soil Science. DOI: http://dx.doi.org/10.1002/jpln.201200077.

Ladd, JN, Foster, RC, Nannipieri, P, Oades, JM. 1996. Soil structure and biological activity. Soil Biochemistry 9: 23–78.

Lara, MJ, McGuire, AD, Euskirchen, ES, Tweedie, CE, Hinkel, KM, Skurikhin, AN, Romanovsky, VE, Grosse, G, Bolton, WR, Genet, H. 2015. Polygonal tundra geomorphological change in response to warming alters future CO2 and CH4 flux on the Barrow Peninsula. Global Change Biology. DOI: http://dx.doi.org/10.1111/gcb.12757.

Lynd, LR, Weimer, PJ, van Zyl, WH, Pretorius, IS. 2002. Microbial cellulose utilization: Fundamentals and biotechnology. Microbiology and Molecular Biology Reviews. DOI: http://dx.doi.org/10.1128/mmbr.66.4.739.2002.

Marx, MC, Kandeler, E, Wood, M, Wermbter, N, Jarvis, SC. 2005. Exploring the enzymatic landscape: Distribution and kinetics of hydrolytic enzymes in soil particle-size fractions. Soil Biology and Biochemistry. DOI: http://dx.doi.org/10.1016/j.soilbio.2004.05.024.

Marx, MC, Wood, M, Jarvis, SC. 2001. A microplate fluorometric assay for the study of enzyme diversity in soils. Soil Biology and Biochemistry. DOI: http://dx.doi.org/10.1016/S0921-8181(01)00079-7.

May, JL, Hollister, RD, Betw ay, KR, Harris, JA, Tweedie, CE, Welker, JM, Gould, WA, Oberbauer, SF. 2020. NDVI Changes show warming increases the length of the green season at Tundra communities in Northern Alaska: A fine-scale analysis. Frontiers in Plant Science 11. DOI: http://dx.doi.org/10.3389/fpls.2020.01174.

McLaren, JR, Buckeridge, KM, van de Weg, MJ, Shaver, GR, Schimel, JP, Gough, L. 2017. Shrub encroachment in Arctic tundra: Betula nana effects on above- and belowground litter decomposition. Ecology. DOI: http://dx.doi.org/10.1002/ecy.1790.

McMillan, JR, Watson, IA, Aii, M, Jaafar, W. 2013. Evaluation and comparison of algal cell disruption methods: Microwave, waterbath, blender, ultrasonic and laser treatment. Applied Energy 103. DOI: http://dx.doi.org/10.1016/j.apenergy.2012.09.020.

Meisel, OH, Dean, JF, Vonk, JE, Wacker, L, Reichart, GJ, Dolman, H. 2021. Porewater δ13CDOC indicates variable extent of degradation in different talik layers of coastal Alaskan thermokarst lakes. Biogeoosciences. DOI: http://dx.doi.org/10.5194/bg-18-2241-2021.

Mueller, CW, Hoessen, C, Steffens, M, Buddenbaum, H, Hinkel, K, Bockheim, JG, Kao-Kniffin, J. 2017. Microscale soil structures foster organic matter stabilization in permafrost soils. Geoderma 293. DOI: http://dx.doi.org/10.1016/j.geoderma.2017.01.028.

Nachtergaele, F. 2001. Soil taxonomy—A basic system of soil classification for making and interpreting soil surveys. Geoderma 99. DOI: http://dx.doi.org/10.1016/S0016-7061(00)00097-5.

Nadelhoffer, KJ, Giblin, AE, Shaver, GR, Laundre, JA. 1991. Effects of temperature and substrate quality on element mineralization in six Arctic soils. Ecology. DOI: http://dx.doi.org/10.2307/1938918.

National Oceanic and Atmospheric Administration’s (NOAA) National Centers for Environmental Information (NCEI). 2020. 1991–2020 U.S. climate normals. Available at https://www.ncei.noaa.gov/access/us-climate-normals/. Accessed 10 May 2021.

Pinheiro, JC, Bates, DM. 2000. Mixed-effects models in S and S-Plus. Springer Science & Business Media. DOI: http://dx.doi.org/10.1109/jasa.2001.s411.

R Core Team. 2019. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computation. Available at http://www.R-project.org.
Rojo, MJ, Carcedo, SG, Mateos, MP. 1990. Distribution and characterization of phosphatase and organic phosphorus in soil fractions. Soil Biology and Biochemistry. DOI: http://dx.doi.org/10.1016/0038-0717(90)90082-B.

Romanovsky, VE, Osterkamp, TE. 2000. Effects of unfrozen water on heat and mass transport processes in the active layer and permafrost. Permafrost and Periglacial Processes. DOI: http://dx.doi.org/10.1002/1099-1530(200007/09)11:3<219::AID-PPP352>3.0.CO;2-3.

Saiya-Cork, KR, Sinsabaugh, R, Zak, DR. 2002. The effects of long term nitrogen deposition on extracellular enzyme activity in an Acer saccharum forest soil. Soil Biology and Biochemistry 34: 1309–1315. DOI: http://dx.doi.org/10.1016/S0038-0717(02)00074-3.

Schaef er, K, Zhang, T, Bruhwiler, L, Barrett, AP. 2011. Amount and timing of permafrost carbon release in response to climate warming. Tellus B: Chemical and Physical Meteorology 63. DOI: http://dx.doi.org/10.1111/j.1600-0889.2011.00527.x.

Scha ure, EAG, Abbott, BW, Bowden, WB, Brovkin, V, Camill, P, Canadell, JG, Chanton, JP, Chapin, FS, Christensen, TR, Ciais, P, Crosby, BT, Czimczik, CI, Gro sse, G, Harden, J, Hayes, DJ, Hugel ei s, G, Jastrow, JD, Jones, JB, Kleinen, T, Koven, CD, Krinner, G, Kuhry, P, Lawrence, DM, McGuire, AD, Natali, SM, O’Donnell, JA, Ping, CL, Riley, WJ, R inke, A, Romanovsky, VE, Sannel, ABK, Sch ädel, C, Schae fer, K, Sky, J, Subin, ZM, Tarnocai, C, Ture tsky, MR, Wal dro p, MP, Walter Anthony, KM, Wickland, KP, Wilson, CJ, Zimov, SA. 2013. Expert assessment of vulnerability of permafrost carbon to climate change. Climatic Change. DOI: http://dx.doi.org/10.1007/s10584-013-0730-7.

Schuur, EAG, McGuire, AD, Schädel, C, Grosse, G, Harden, JW, Hayes, DJ, Hugelius, G, Koven, CD, Kuhry, P, Lawrence, DM, Natali, SM, Olefeldt, D, Romanovsky, VE, Schaefer, K, Turetsky, MR, Treat, CC, Vonk, JE. 2015. Climate change and the permafrost carbon feedback. Nature. DOI: http://dx.doi.org/10.1038/nature14338.

Seifert-Mon son, LR, Hill, BH, Kolka, RK, Jicha, TM, Leht o, LL, Elonen, CM. 2014. Effects of sulfate deposition on pore water dissolved organic carbon, nutrients, and microbial enzyme activities in a northern peatland. Soil Biology and Biochemistry 79. DOI: http://dx.doi.org/10.1016/j.soilbio.2014.09.007.

S haver, GR, Gib lin, AE, Nadel hoffer, KJ, Thieler, KK, Downs, MR, Laundre, JA, Rastetter, EB. 2006. Carbon turnover in Alaskan tundra soils: Effects of organic matter quality, temperature, moisture and fertilizer. Journal of Ecology 94. DOI: http://dx.doi.org/10.1111/j.1365-2745.2006.01139.x.

S insabaugh, RL. 2010. Phenol oxidase, peroxidase and organic matter dynamics of soil. Soil Biology and Biochemistry 42: 391–404. DOI: http://dx.doi.org/10.1016/j.soilbio.2009.10.014.

S insabaugh, RL, Gallo, ME, Lauber, C, Wal drop, MP, Zak, DR. 2005. Extracellular enzyme activities and soil organic matter dynamics for northern hardwood forests receiving simulated nitrogen deposition. Biogeochemistry 75. DOI: http://dx.doi.org/10.1007/s10533-004-7112-1.

Sinsabaugh, RL, Saiya-Cork, K, Long, T, Osgood, MP, Neher, DA, Zak, DR, Norby, RJ. 2003. Soil microbial activity in a Liquidambar plantation unresponsive to CO2-driven increases in primary production. Applied Soil Ecology. DOI: http://dx.doi.org/10.1016/S0929-1393(03)00002-7.

Skujins caron, J, Burns, RG. 1976. Extracellular enzymes in soil. Critical Reviews in Microbiology. DOI: http://dx.doi.org/10.3109/10408417609102304.

Soil Survey Staff. 1999. Soil taxonomy; A basic system of soil classification for making and interpreting soil surveys. 2nd ed. Washington, DC: Natural Resources Conservation Service (U.S. Department of Agriculture Handbook No. 436).

Steinweg, JM, Dukes, JS, Wallenstein, MD. 2012. Modeling the effects of temperature and moisture on soil enzyme activity: Linking laboratory assays to continuous field data. Soil Biology and Biochemistry. DOI: http://dx.doi.org/10.1016/j.soilbio.2012.06.015.

Stemmer, M, Gerzak e b, MH, Kandeler, E. 1998. Organic matter and enzyme activity in particle-size fractions of soils obtained after low-energy sonication. Soil Biology and Biochemistry. DOI: http://dx.doi.org/10.1016/S0038-0717(97)00093-X.

Sulikowska, A, Walawender, J, Walawender, E. 2019. Temperature extremes in Alaska: Temporal variability and circulation background. Theoretical and Applied Climatology. DOI: http://dx.doi.org/10.1007/s00704-018-2528-z.

Tarnocai, C. 2009. The impact of climate change on Canadian peatlands. Canadian Water Resources Journal. DOI: http://dx.doi.org/10.4296/cwrij3404453.

Treseder, KK, Vitousek, PM. 2001. Effects of soil nutrient availability on investment in acquisition of N and P in Hawaiian rain forests. Ecology. DOI: http://dx.doi.org/10.1890/0012-9658(2001)082[946:EOSNAJ]2.0.CO;2.

Villarreal, S, Hollister, RD, Johnson, DR, Lara, MJ, Webber, PJ, Tweedie, CE. 2012. Tundra vegetation change near Barrow, Alaska (19722010). Environmental Research Letters. DOI: http://dx.doi.org/10.1088/1748-9326/7/1/015508.

Wallenstein, MD, Burns, RG. 2015. Ecology of extracellular enzyme activities and organic matter degradation in soil: A complex community-driven process, in Methods of soil enzymology. DOI: http://dx.doi.org/10.2136/sssabookser9.c2.

Wallenstein, MD, Weintraub, MN. 2008. Emerging tools for measuring and modeling the in situ activity of soil extracellular enzymes. Soil Biology and Biochemistry. DOI: http://dx.doi.org/10.1016/j.soilbio.2008.01.024.
Walz, J, Knoblauch, C, Bohme, L, Pfeiffer, EM. 2017. Regulation of soil organic matter decomposition in permafrost-affected Siberian tundra soils—Impact of oxygen availability, freezing and thawing, temperature, and labile organic matter. Soil Biology and Biochemistry 110. DOI: http://dx.doi.org/10.1016/j.soilbio.2017.03.001.

Weintraub, MN, Schimel, JP. 2003. Interactions between carbon and nitrogen mineralization and soil organic matter chemistry in Arctic tundra soils. Ecosystems. DOI: http://dx.doi.org/10.1007/s10021-002-0124-6.

Zhang, Q, Zhou, W, Liang, G, Sun, J, Wang, X, He, P. 2015. Distribution of soil nutrients, extracellular enzyme activities and microbial communities across particle-size fractions in a long-term fertilizer experiment. Applied Soil Ecology 94. DOI: http://dx.doi.org/10.1016/j.apsoil.2015.05.005.

Zhang, T, Stamnes, K. 1998. Impact of climatic factors on the active layer and permafrost at Barrow, Alaska. Permafrost and Periglacial Processes 9. DOI: http://dx.doi.org/10.1002/(sici)1099-1530(19980709)9:3<229::aid-ppp286>3.0.co;2-t.

Weintraub, MN, Schimel, JP. 2003. Interactions between carbon and nitrogen mineralization and soil organic matter chemistry in Arctic tundra soils. Ecosystems. DOI: http://dx.doi.org/10.1007/s10021-002-0124-6.

Zhang, T, Stamnes, K. 1998. Impact of climatic factors on the active layer and permafrost at Barrow, Alaska. Permafrost and Periglacial Processes 9. DOI: http://dx.doi.org/10.1002/(sici)1099-1530(19980709)9:3<229::aid-ppp286>3.0.co;2-t.

Weintraub, MN, Schimel, JP. 2003. Interactions between carbon and nitrogen mineralization and soil organic matter chemistry in Arctic tundra soils. Ecosystems. DOI: http://dx.doi.org/10.1007/s10021-002-0124-6.

Zhang, T, Stamnes, K. 1998. Impact of climatic factors on the active layer and permafrost at Barrow, Alaska. Permafrost and Periglacial Processes 9. DOI: http://dx.doi.org/10.1002/(sici)1099-1530(19980709)9:3<229::aid-ppp286>3.0.co;2-t.

Zhang, T, Stamnes, K. 1998. Impact of climatic factors on the active layer and permafrost at Barrow, Alaska. Permafrost and Periglacial Processes 9. DOI: http://dx.doi.org/10.1002/(sici)1099-1530(19980709)9:3<229::aid-ppp286>3.0.co;2-t.