

GLACIAL EFFECTS ON STREAM WATER NITRATE:
AN EXAMINATION OF PAIRED CATCHMENTS IN SOUTHERN MONTANA

by

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ABSTRACT

Nitrogen is frequently a limiting nutrient in biologic systems. Previous research on alpine streams and lakes in the Beartooth Mountains, Montana/Wyoming has demonstrated nitrate concentrations in waters draining glaciated catchments that are up to ten times greater than comparable adjacent non-glaciated catchments. The enhanced nitrate concentrations in the glacial fed lakes have been associated with increased diatom abundance relative to the snow fed-lakes. However, the source of the enhanced nitrate input remained undetermined, as well as how nitrate concentrations vary temporally during summer melt.

This study measured concentrations of nitrate and ammonium and the isotopic composition of nitrate over the 2016 melt-season in a paired catchment system, in the Beartooth Mountains, Montana. The two catchments have similar elevations, atmospheric inputs, bedrock geology, area, and contain lakes, however, one catchment contains a glacier, the other does not. The stream waters in the glaciated catchment showed significantly elevated nitrate concentrations relative to those in the non-glaciated catchment and to catchment atmospheric input, as determined by snowpack nitrate concentrations. Nitrate concentrations in the glacial stream were observed to increase both temporally as the melt-season progressed, and spatially, with distance downstream from the glacier terminus. Ammonium concentrations in the glacial stream were highest close to the glacier terminus, declining with distance downstream, but also increasing during the melt season. Nitrate isotopic values distinguish the stream waters from atmospheric inputs indicating additional nitrate sources in the catchment. Potential additional sources include inorganic nitrogen released from bedrock sources and microbially fixed nitrogen.

Abiotic laboratory weathering experiments simulating subglacial conditions reacted deionized water with finely milled bedrock at 4°C, and a modest quantity of ammonium was released. Potassium is often replaced by ammonium in minerals. Rocks from the study area contained ~3% potassium by weight. Ammonium could then be converted to nitrate through microbial processes within the proglacial environment adding to the atmospheric nitrate input to the stream nitrate budget. However, estimated rates of sediment production, and by inference ammonium production, cannot account for the observed nitrate concentrations and flux, indicating an additional nitrate source, which is most likely ultimately derived from microbial nitrogen fixation.

INTRODUCTION

General Overview

Nitrogen (N) is a critical component to all life and it is often not found in readily assimilated forms (e.g. nitrate, nitrite, and ammonium) in many environments, therefore N commonly acts as a limiting nutrient (Gruber & Galloway, 2008). The effects of glacial processes on N-cycling is an understudied aspect of biogeochemical research. For example, few studies have explicitly addressed the importance of weathering as a N source across alpine and headwater catchments (Montross et al., 2013a) despite the fact they are often nutrient limited. However, Saros et al. (2010) found that in catchments in the northern Rockies, lakes fed by snowpack and glacial meltwaters (GSF lakes), often contained ten (and up to 200) times the amount of nitrate when compared to similar adjacent lakes fed only by snowpack meltwaters (SF lakes). Further, Slemmons et al. (2015) in a paired catchment study in the Beartooth Mountains, MT inferred that differences in the nitrate input to the GSF Jasper Lake, impacted diatom populations relative to the neighboring, SF Albino Lake over a period of ~1000 years. Slemmons et al., (2015) noted that over the past 1000 years Jasper Lake sediments contained diatom assemblages that required higher concentrations of N when compared to those in sediments in the adjacent Albino Lake. This strongly indicates that in these high Alpine systems, in the Beartooth Mountains, biologically available N is an important ecologic driver affecting the diversity of primary producers and their overall biomass.

Nitrate enrichment has not always been observed when comparing neighboring SF and GSF lakes, for example in the north Cascades, WA (Williams et al., 2016). The lack of enhanced nitrate concentrations in the GSF lakes relative to SF lakes in the north Cascades compared to the observations in the Beartooth Mountains is not fully understood (Williams et al., 2016). However, differences in (i) precipitation, 1000-4000mm/year in the Cascades vs 681mm/year during the 2015-2016 water year at Beartooth Lake, and/or (ii) underlying bedrock, diverse bedrock material over a larger geographic region in the north Cascades vs dominantly granitic gneiss in Beartooth Mountains catchments (Slemmons et al., 2015), have been suggested as potential causal factors for the differing patterns of nitrate abundance in GSF and SF lakes in the two mountains ranges (Williams et al., 2016). The source(s) of the elevated nitrate inputs to the GSF lakes in the Beartooth Mountains remains unknown and determining these sources and how they may vary during a summer melt-season is the goal of this research. Potential N sources to the catchment are from the bedrock and atmosphere directly and also through microbial nitrogen fixation. Nitrate can be deposited directly to the catchment from the atmosphere but may also be the product of some combination of physical, chemical and microbial processes that modify other catchment sources of N.

Rationale and Background Information

N in rocks

Rocks and sediments can be a significant reservoir of N in many environments, but, N-bearing rocks have historically been overlooked as N reservoirs, as N

concentrations were assumed to be insignificant and were therefore not measured (Holloway & Dahlgren, 2002; Houlton et al., 2018). Recent research has shown that bedrock N inputs from weathering are of a similar magnitude to atmospheric inputs in many terrestrial environments (Houlton et al., 2018). On a global scale, ~60% of N is stored as minerals or melt in the mantle, with another ~10.5% stored in the crust (see Table 1.1) (Bebout et al., 2016; Li et al., 2007; Palya et al., 2011). It is possible for NH_4^+ to be incorporated into common rock forming minerals such as biotite and orthoclase by replacing for K^+ in crystal lattices, (Boyd et al., 1993; Busigny et al., 2004) as well as in some naturally N-rich, but relatively rare, minerals (e.g. buddingtonite, ammonioleucite, mundrabillaite) (Holloway and Dahlgren, 2002). Boyd et al., (1993) documented granitic rocks with NH_4^+ concentrations above 170 ppm and individual minerals (orthoclase) with concentrations of 900 ppm. Geologic N inputs from weathering diverse lithologies may exceed biotic demands in some alpine environments (e.g. the Sierra Nevada of California) (Holloway and Dahlgren, 1999). Furthermore, elevated $\delta^{15}\text{N}$ ($\sim +11$ ‰) values in metamorphic rocks (vs ~ 0 ‰ in atmospheric N_2) have been shown to indicate a metasedimentary source (through anatexis) for the N, highlighting that distinct sources of N can carry distinct isotopic signatures (Boyd et al., 1993) (Figure 1.1); other processes may also enact significant isotopic signatures (e.g. N_2 fixation) (Högberg, 1997) and this signature is not indicative of a specific source without supporting evidence.

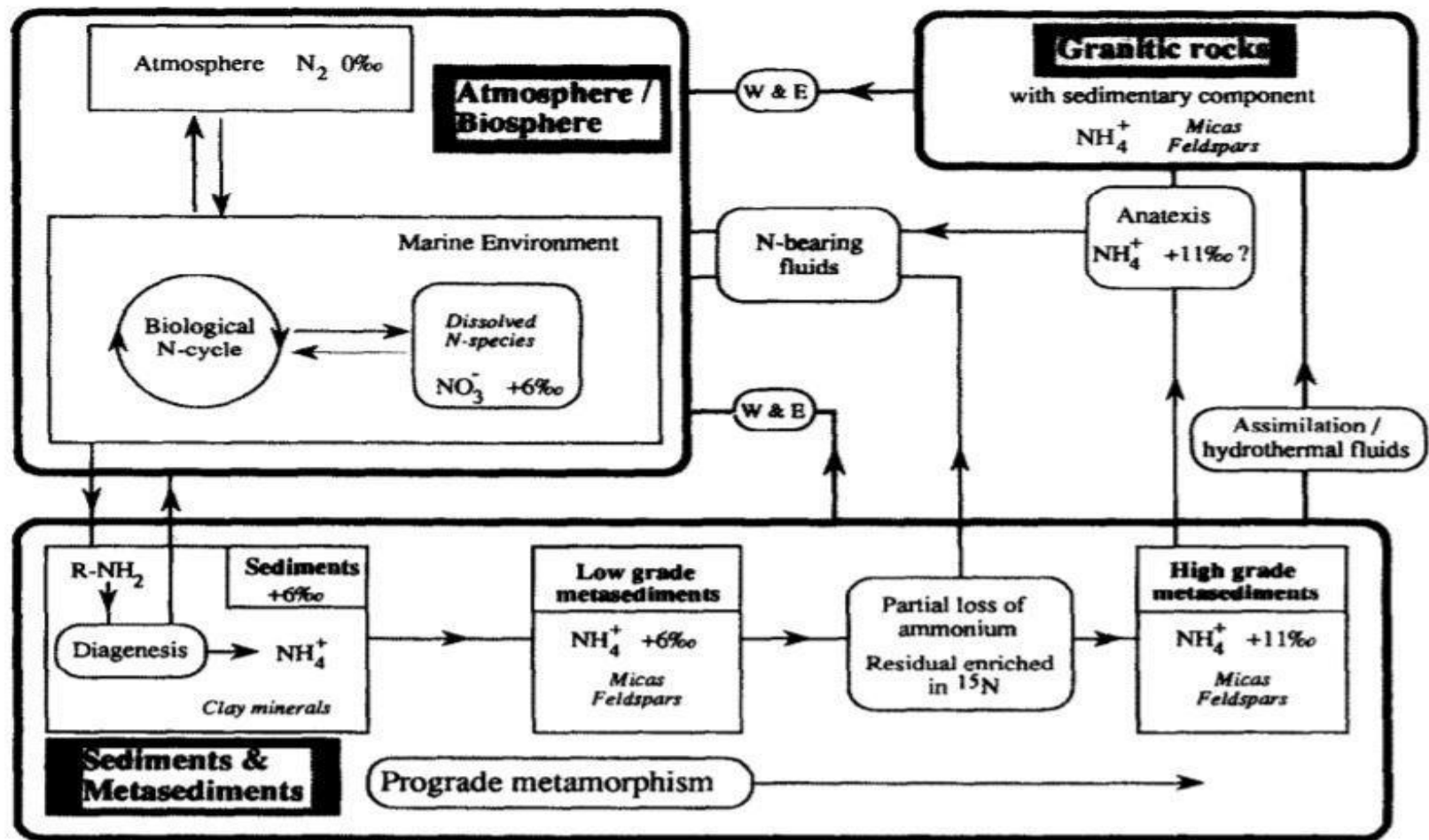


Figure 1.1. Isotopic fractionation processes occurring in the N-cycle from Boyd et al., 1993. ‰ values are for $\delta^{15}\text{N}$ of the various compounds. Reprinted from *Geochimica et Cosmochimica Acta*, Volume 57, Boyd et al., The measurement of $\delta^{15}\text{N}$ in crustal rocks by static vacuum mass spectrometry: Application to the origin of the ammonium in the Cornubian batholith, southwest England, pg. 9, 1993 with the permission of Elsevier.

Table 1.1. Global N budget from: Bebout et al., 2016, Li et al., 2007, and Palya et al., 2011 (and sources referenced therein) showing reservoir distribution of N on the planet.

Source	Reservoir size 10^{21} g	% in reservoir
Atmosphere	3.9	29.3
Continental crust	1.1	8.3
Oceanic crust	0.32	2.4
Mantle	7.97	60
Totals	13.29	100

Glacial Processes

Glaciers are effective drivers of physical erosion. The amount of sediment produced in glaciated catchments varies up to four orders of magnitude depending on thermal regime and underlying geology (Hallet et al., 1996); in temperate thermal regimes sediment production can be up to ten times greater than that from comparable unglaciated catchments (Anderson, 2007). The majority of comminution of glacially transported material occurs at the glacier bed (Boulton, 1978). Glacial basins produce amounts of fine grained sediment higher than the global average (Gurnell et al., 1996) and these smaller particles have high surface areas and therefore increased potential for surface reactions (chemical or biological) (Brown et al., 1996). Anderson (2007), notes that the high mineral surface area generated during glacial reworking is why subglacial chemical weathering rates have been identified as significant players in global geochemical cycles. These processes can potentially lead to the production of dissolved species (e.g. iron, thiosulfate, hydrogen) in glacial systems that could serve as an energy source for microbial processes (Boyd et al., 2014; Telling et al., 2015).

Atmospheric Deposition

Atmospheric deposition of N species (ammonium, nitrite and nitrate) is important to account for, if N cycling in alpine environments is to be fully understood. Wasiuta et al., (2015a) examined local and regional sources of atmospheric N deposition in two adjacent glaciated catchments in the southern Canadian Rockies over the 2008/2009 water year via measurements of snowpack and summer precipitation. Ammonium, nitrite, and nitrate concentrations were all higher in rain events than in snow events by a factor of two (Wasiuta et al., 2015a). Concentrations of all N species were found to decrease slightly with elevation in both rain and snow events, but the overall quantity of N deposition increased with elevation due to greater volumes of precipitation with increasing elevation (Wasiuta et al., 2015a). In low deposition, dry environments (e.g. Antarctica), loss of nitrate from the snowpack through photolysis has been documented (e.g. France et al., 2011 and references therein; Neubauer and Heumann 1988), but in high deposition environments such as the Beartooth Mountains this process is likely of minimal impact (Buffen et al., 2014).

Stable Isotope Chemistry of Nitrogen and Oxygen in Nitrate

The stable isotopic composition of the N and O in nitrate has been used to determine atmospheric sources of nitrate in the Atacama (Michalski et al., 2004) the Antarctic (Savarino et al., 2007), and Alberta and British Columbia (Wasiuta et al., 2015b). Isotopic values are typically expressed using the δ notation,

$$\delta = ((R_{\text{Sample}}/R_{\text{Standard}}) - 1) \times 1000 \quad (\text{Equation 1})$$

where R is the ratio of the minor isotope over the major isotope. The multiplier (1000) is used due to the typically small ratios of minor to major isotopes; and therefore, δ values are reported in per mille (‰). Atmospheric N_2 is defined as the standard for measuring N isotopes, with a $\delta^{15}N$ value of 0 ‰ (Figure 1.1); similarly, Vienna Standard Mean Ocean Water (VSMOW) is defined as the standard for oxygen isotopes in water with a $\delta^{18}O$ value of 0 ‰ (<http://www.ciaaw.org>).

Wasiuta et al., (2015b) examined the $\delta^{15}N$ and $\delta^{18}O$ values of nitrate in a companion study to that described above, in the “Atmospheric Deposition” section (Wasiuta et al., 2015a), in an attempt to understand sources for inorganic N species deposition in the adjacent glaciated catchments. Using the known differences in sources’ isotopic ($\delta^{18}O$ and $\delta^{15}N$) signatures of nitrate, Wasiuta et al., (2015b), were able to attribute some of the nitrate deposited in their study area to local, anthropogenic sources, when coupled with modelled air mass trajectories. For example, oxidation pathways in the atmosphere produce elevated nitrate $\delta^{18}O$ values of 51-101‰, compared with industrial and urban sources yielding nitrate $\delta^{18}O$ values of 17-35‰, or elevated $\delta^{15}N$ being associated with relatively clean air (and lower nitrate concentrations) as is seen in pre-industrial ice cores (Freyer et al., 1996)). They also detected significant differences in nitrate sources between adjacent catchments and along elevational gradients. Topography could influence where the deposition of reactive N would occur, based on the trajectory of incoming precipitation, shielding higher sites from local and regional sources of reactive N (Wasiuta et al., 2015a).

Michalski et al., (2003) developed a method of measuring $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$ values in nitrate. Comparisons of these two minor oxygen isotopes can be made using the Δ notation, where

$$\Delta^{17}\text{O} \sim \delta^{17}\text{O} - 0.52 \delta^{18}\text{O} \quad (\text{Equation 2})$$

Most terrestrial, meteoric, and oceanic sources of oxygen have a $\Delta^{17}\text{O}$ of approximately 0 (Miller, 2002). Deviations from this value occur when mass fractionation in chemical reactions occur at different rates than described in Equation 2. Formation of ozone is one such reaction, where the incorporation of different isotopes of oxygen seems to be nearly independent of the mass of these isotopes – i.e. a mass independent process. This leads to $\Delta^{17}\text{O}$ values of 20-50‰ in ozone (Thiemens and Heidenreich, 1983). Comparison of $\delta^{18}\text{O}$ and $\Delta^{17}\text{O}$ values in nitrate allows determination of initial source reservoirs of the oxygen atoms in nitrate, including those generated via mass independent processes (Michalski et al., 2003). Michalski et al., (2004) used this method to determine that ~100% of the nitrate ore (used primarily in fertilizer) in the Atacama, is derived from atmospheric deposition, which is almost entirely dry deposition in this setting.

Microbial Processes

Basal ice of glaciers, subglacial sediments, and subglacial meltwaters have been shown to harbor microbial communities (glacial ice and meltwaters showed bacterial concentrations of $5.3 \times 10^4 \text{ mL}^{-1}$ to $5.9 \times 10^7 \text{ mL}^{-1}$) (Sharp et al., 1999), which have been shown to be active (e.g. ATP concentrations, stable isotopic values, RNA based

evidence) (Boyd et al., 2014; Boyd et al., 2011; Christner et al., 2014; Foght et al., 2004; Gaidos et al., 2004; Hamilton et al., 2013; Sharp et al., 1999). Further, the concentration of microbes has been shown to increase with the concentration of sediment found in the basal ice (Sharp et al., 1999). Microbial processes have been demonstrated to increase mineral weathering rates in laboratory experiments simulating subglacial processes using subglacial sediments and waters from Haut Glacier d'Arolla, Switzerland and Bodalsbreen Norway (Montross et al. 2013). Montross et al., (2013) also showed biotic reduction of nitrate (with brief nitrite accumulations), under both oxic and anoxic initial conditions, using waters and sediments taken from the outlets of subglacial streams, thus indicating that microbes from subglacial streams are capable of influencing N cycling.

Bedrock mineralogy may drive microbial community make up (Mitchell et al., 2013; Skidmore et al., 2005). Subglacial environments on Ellesmere Island, Canadian Arctic and of the Taylor Glacier, Antarctica have been shown to be viable microbial habitats where water is present (Skidmore et al., 2000; Montross et al. 2014). N in the form of ammonium was measured in the Antarctic basal ice, having the highest concentrations in ice that contained greater than 1% sediment; this is also where cell concentrations were highest (1.2×10^4 cells g^{-1}) (Montross et al., 2014). Furthermore, microbial processes have been inferred to affect gas chemistry of basal ice, with CO_2 production occurring in presumed microbial habitats (debris rich ice). CO_2 production in this environment cannot be explained with simple refreezing and likely indicates active microbial respiration (Montross et al., 2014).

Increased levels of sub-glacially derived nitrate relative to atmospheric sourced waters have been observed in certain glacial environments (Wynn et al., 2007). Wynn et al. (2007) reported that subglacial waters from Midtre Lovénbreen, Svalbard not only contained high levels (between two and five times) of nitrate when compared to supraglacial (atmospherically derived) waters, but that the nitrate isotopic composition based on $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values, was indicative of it being derived from microbial processes. The nitrate flux in the subglacially sourced streams were also found to be higher than the amount deposited via atmospheric processes, indicating that the nitrate had an organic or geologic source (Wynn et al., 2007).

Microbial communities capable of both nitrification and nitrate reduction in subglacial sediments have been found at Robertson Glacier, Alberta, Canada (Boyd et al., 2011). Quantitative PCR was performed to estimate the number of archaeal and bacterial functional gene templates involved in nitrification, nitrate reduction, and nitrogen fixation. Bacterial nitrifiers were found to have a greater diversity than archaeal nitrifiers, despite archaeal predominance in brackish (Bernhard et al., 2007) and nutrient poor environments (Mosier and Francis, 2008). Microcosm laboratory experiments were performed with native subglacial sediments and a salts media mimicking environmental porewater concentrations with minimal nutrient amendments to determine the potential for nitrification, nitrate reduction, N_2 fixation in the subglacial sediments. Evidence of nitrification in microcosm experiments had a lag time (17-105 days) when compared to marine samples (1-3 days), which is thought to be indicative of lower basal levels of nitrification in the subglacial environment (Boyd et al., 2011). Similarly, nitrate reducers

were present and seen to be active after a lag time (18 days incubation), again indicating low basal levels of nitrate reduction in the subglacial environment (Boyd et al., 2011).

Despite evidence for genes capable of nitrogen fixation, and an environment where organic N is depleted relative to carbon, N_2 fixation was not detected (Boyd et al., 2011).

Melt waters sourced from rock glaciers in the front range of Colorado and Wyoming were analyzed by Williams et al. (2007). While differences have been documented between rock glaciers and glaciers, characteristics specific to mountain ranges have been shown to play a large role in physical, biologic, and chemical characteristics as well (Fegel et al., 2016). Nitrate concentrations from the Green Lake 5 rock glacier (in the Green Lakes Valley) followed a “trough” like trend seasonally each year as the melt-season progressed (June-October), starting at concentrations of $\sim 50\mu\text{M}$, then decreasing to $\sim 30\mu\text{M}$, before increasing again to $\sim 80\mu\text{M}$. Nitrate concentrations also tended to be higher by $\sim 30\text{-}50\mu\text{M}$ during drought years. At all seven rock glaciers, streams had higher nitrate concentrations (approximately double) than reference streams taken from the same catchments. Ammonium concentrations were low ($<5\mu\text{M}$) at all sites throughout the melt-season with the exception of waters taken from the terminus of an active glacier (not a rock glacier). The ammonium concentrations in the waters from the active glacier mirrored nitrate concentrations “trough” pattern, starting the melt-season at $\sim 25\mu\text{M}$, before decreasing to $\sim 5\mu\text{M}$, and then gradually increasing to $\sim 20\mu\text{M}$ by the end of the melt-season. Early elevated concentrations of nitrate at all sites, and for ammonium at the glacier terminus site, were interpreted to be ionic pulses from concentrated meltwater of snowpack (Williams et al., 2007). Fluorescence characteristics of dissolved

organic material were also analyzed in the soils on the rock glaciers; the fluorescence index found at the end of the melt-season was similar to those found in Antarctica produced by aquatic organisms (Cory and McKnight, 2005). Other research in the Green Lakes Catchment had shown microbial populations that were active throughout winter months in dark, cold, oligotrophic environments (Ley et al., 2004) and that environment was limited in carbon, which resulted in the system moving towards net nitrification (Williams et al., 1997). This data, taken together, indicates that microbial populations at RG5 in the Green Lakes Valley, are likely responsible for elevated nitrate concentrations (Williams et al., 2007). Other research in the Colorado Rockies has shown that nitrification does not occur in snowpack, in the absence of finely comminuted sediment, indicating that the presence of ammonium alone (in the snowpack) may not fulfill the necessary conditions for nitrification (Williams et al., 1996).

Microbial nitrification has been inferred based on the isotopic composition of nitrate in stream waters in glacial and permafrost environments (Louiseize et al., 2014). In paired catchment studies of undisturbed and disturbed permafrost elevated levels of nitrate in streams in the disturbed permafrost were an order of magnitude higher than undisturbed permafrost streams. Isotopic analysis of the nitrate has been used to infer that the elevated nitrate was derived from microbial nitrification; with (i) more negative $\delta^{18}\text{O}$ values in disturbed permafrost derived streams than undisturbed permafrost from June to July and (ii) $\delta^{15}\text{N}$ values showing the nitrate source changed from atmospheric (80-100%) during the first outing of the season (early June), to N derived from mineral sources (55-100%) for the rest of the melt-season (through the end of July) (Louiseize et al., 2014).

Broader Implications of Glacial Processes Affecting Stream Water N Concentrations

The role of glacial weathering processes in mountainous catchments on the N-cycle has largely been overlooked. This study aims to investigate the role of glacial processes on increased nitrate concentrations in glacial catchments where these increased nitrate concentrations have been linked to primary production in lakes directly fed by the glacial meltwater (Slemmons et al., 2015). The enhanced nitrate in the glacially fed system can be traced downstream from the headwater lake into those fed from its outflow, also modifying populations of primary producers in these downstream lakes (Slemmons et al., 2015). Nitrification as an important microbial process in glacial and subglacial environments has only recently been considered; and thus gaining a greater understanding of the magnitude of this process and the potential controlling variables is key to understanding the N – cycle in these systems (Boyd et al., 2011).

Hypothesis

Finely comminuted bedrock from recently glaciated substrate in the Beartooth Mountains, MT, provides increased fresh surface area to supply ammonium which leads to elevated levels of nitrate in glacial meltwaters.

To test this hypothesis three objectives were undertaken 1) determine the amount of water soluble N released from finely comminuted bedrock under simulated glacial conditions, 2) determine the proportion of atmospheric nitrate in the stream waters in each catchment using isotope mass balance techniques; the nitrate concentration and isotopic composition of atmospheric input will be determined from snowpack samples,

and 3) undertake biotic (with native microbes) and abiotic weathering experiments in the laboratory using sediments from the catchments and waters to determine the potential magnitude of biologic nitrate production potential.

METHODS

Study Area

Overview

The field area for this research lies in the Absaroka-Beartooth Wilderness of southern Montana and northern Wyoming. It was designated a Wilderness area in 1978 and has nearly one million (937,032) acres of largely pristine land according to Wilderness Connect (<http://www.wilderness.net/NWPS/wildView?WID=1>). The Wilderness area is part of the Greater Yellowstone Ecosystem (GYE) and has some of the most expansive areas over 3000m in the contiguous United States. According to Glaciers of the American West survey carried out in 2011 (<http://glaciers.us/glaciers-montana-2.html>) the Wilderness contains 401 glaciers and snowfields, based on mapping photography from 1958-1982. The Wilderness area includes a significant proportion (35%) of all glaciers and snowfields in Montana. In addition to snow fields and glaciers the Wilderness also contains some 660 rock glaciers (Seligman, 2009). The study area lies in the southeast portion of the Wilderness, just north of the Wyoming border (Figure 2.1).



Figure 2.1. Showing the study area located just northeast of Yellowstone National Park. Base map from ESRI.

Geology

The Beartooth Mountains are composed primarily of Archean basement rock though there is some Paleozoic sedimentary cover on the flanks of the mountains and in isolated areas that resisted erosion after the mountains began being uplifted (Henry and Mogk, 2003). The Beartooth Mountains formed during a Laramide style orogeny resulting in the uplift and exposure of large expanses of the Wyoming Craton. The basement rocks have been dated to 2.74-2.8Ga and are thought to consist mainly of intrusive granitoids with some sedimentary inclusions (Henry and Mogk, 2003). Many

intrusions occurred throughout the history of the region, with major dike swarms occurring at 1.3 Ga and 780 Ma (Henry and Mogk, 2003). Laramide uplift began in the late Cretaceous-early Cenozoic (65-57 Ma) with the vast majority of sedimentary rocks being shed in the following few million years (Henry and Mogk, 2003). The bulk of the field area is mapped as being dominantly granitic gneiss and migmatite which comprises alternating bands of more felsic and more mafic gneiss; and with some inclusions of metasedimentary and metaigneous rocks. (Van Gosen et al., 2000). The bedrock geology of the two catchments are composed of granitic gneiss (trondhjemite in chemical composition), with more mafic dikes scattered throughout Wilderness Area, though only one was confirmed in the field area; the dike is mapped as “alkali olivine dolerite, metadolerite, metanorite, metagabbro, and quartz dolerite, and dikes of uncertain or unknown affinities” (Van Gosen et al., 2000).

Glacial History of the Beartooth Mountains

The high elevation region of the Beartooth Mountains has been extensively influenced by glaciers during the Pleistocene. Ice covering the Beartooth Mountains fed into the Yellowstone Ice Cap (Licciardi and Pierce, 2018; Pierce, 1982), as well as supplying outlet glaciers to the north. The Beartooth Plateau contributed about half of the ice that flowed out into Paradise Valley from the Yellowstone Ice Cap during the height of the Pinedale glaciation (the last glacial maximum, defined as between 19.8 and 15.5 ka in the Greater Yellowstone Glacial System (GYGS)), and up to 75% once lower elevation ice on the Yellowstone Plateau began to retreat (Licciardi and Pierce, 2018; Pierce,

1982). The Beartooth Uplift is a high elevation region that receives significant precipitation due to orographic uplift, when compared to surrounding areas; in the early Pinedale Glaciation the Beartooth Uplift would have acted as one of the source regions for accumulating ice as the GYGS began to form (Licciardi and Pierce, 2018). As Ice began to accumulate on the Yellowstone Plateau, precipitation from prevailing easterly moving storms would have been preferentially deposited on the western side of the building ice mass due to orographic uplift; this in turn would have decreased the available moisture to be deposited in the Beartooth Uplift which in turn caused the early (19.8 ka) retreat of the Clarks Fork Outlet Glacier 45km to Crandall Creek (18.2 ka), though the Beartooth Plateau remained glaciated at least through 15 ka when the GYGS separated in the north, marked by the Junction Butte Moraines (Licciardi and Pierce, 2018). Similarly, during the Bull Lake glaciation (penultimate glacial maximum, with a mean glacial maximum age of 150 ± 4 ka the Beartooth Plateau likely provided a large component of the GYGS (Licciardi & Pierce, 2018)), though features preserving this contribution from the plateau would have been scoured by the following Pinedale Glaciation. Bull Lake moraines in the western edge of the Wilderness Area (near Cowen Peak) have been preserved and only extend about 5% farther down drainage than the Pinedale moraines, so it is likely that the Bull Lake and Pinedale Glaciations were of a similar magnitude in this region. The study area would have been almost entirely covered by ice during both the Pinedale and Bull Lake glacial events, with only the highest peaks forming nunataks (Figure 2.2). Retreat of the Yellowstone Ice Cap during the Pinedale Glaciation is thought to have started between 19.8 and 15.5 KA based on ^{10}Be ages derived from

terminal moraines near the Clarks Fork of the Yellowstone River and Pinedale 2 (located between Jackson Lake and Jenny Lake) respectively (Licciardi and Pierce, 2018). The large expanse of high altitude land in the Beartooth Mountains served as a refuge for glacial ice after much of the Yellowstone Ice Cap was in retreat, and substantial amounts of ice likely persisted for significant time after regional retreat began (Pierce 1982).

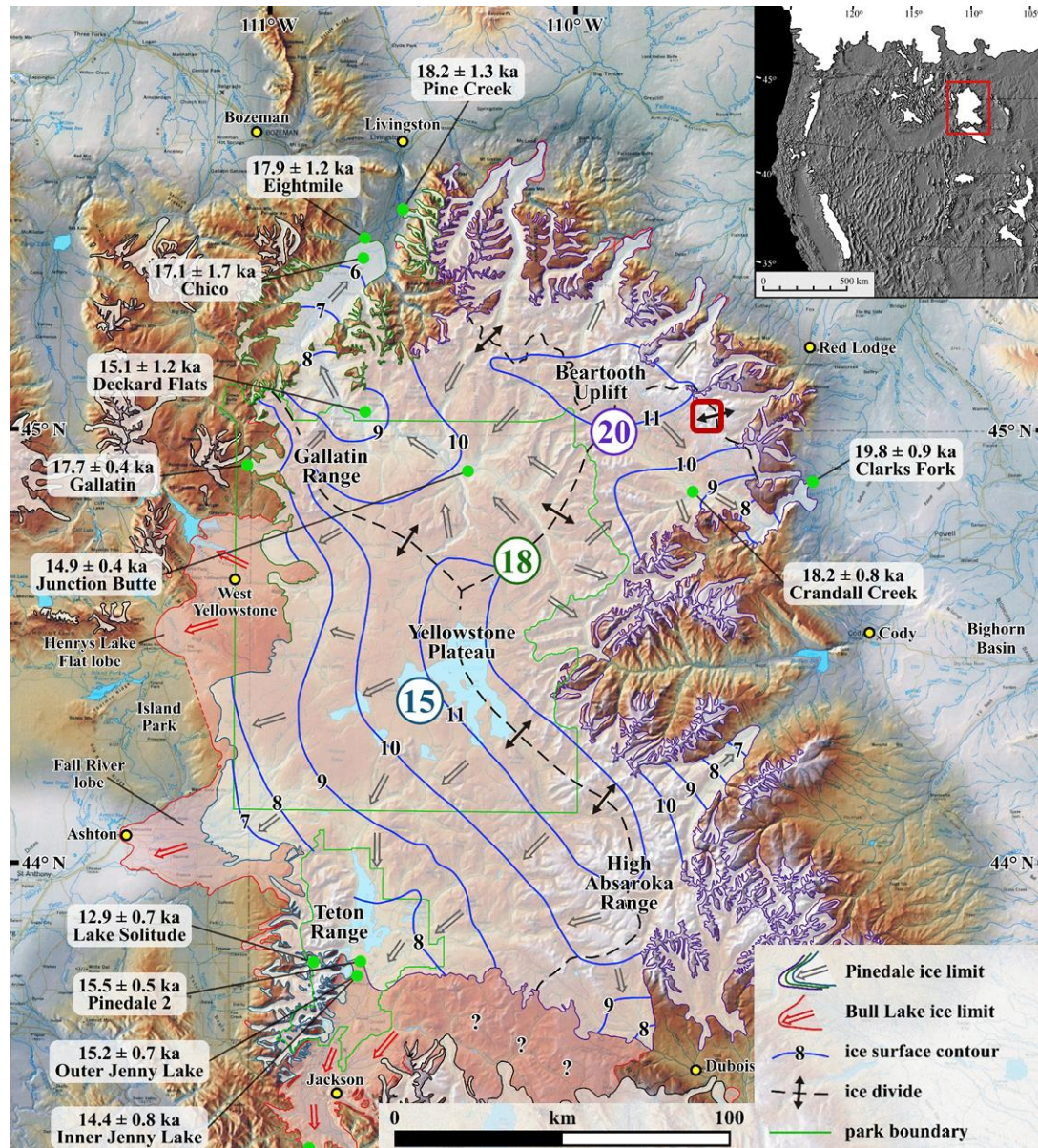


Figure 2.2. Glacial history map showing the extent of the Bull Lake and Pinedale glaciations of the Greater Yellowstone Glacial System (GYGS). Surface contours on the maximum extent of Pinedale ice are in thousands of feet. Pinedale ice margin line colors correspond with estimates for maximum ages around the periphery of the GYGS: purple – early Pinedale (~22–18 ka); green – middle Pinedale (~18–16 ka); blue – late Pinedale (~16–13 ka). Circles enclosing ages in ka schematically depict southwest migration of the center of ice mass through time. Dashed lines indicate uncertain or approximate ice limits. Field area is shown in red square to the east of the northern boundary of Yellowstone National Park. Reprinted from Quaternary Science Reviews, Volume 200, Licciardi and Pierce, History and dynamics of the Greater Yellowstone Glacial System during the last two glaciations, pg. 2, 2018, with permission from Elsevier.

During the Holocene, since 11.7ka, six specific cold periods have been documented in the Northern Hemisphere (Wanner et al., 2011). However, the intensity of each cold period for any given region (e.g. the Beartooth Mountains) varies greatly, such that some may lead to mountain glacier growth and advance, but others have limited impact (Wanner et al., 2011). Graf (1971), describes geomorphic evidence, for two neoglacial advances, glacial advances that have occurred since the end of Pinedale Glaciation in the upper Rock Creek drainage in the Beartooth Mountains, which lies immediately east of the study area. Dating control on these features is poor, but Graf, (1971) tentatively assigns dates of 2800 and 400 year BP, but noting that the margin of error could be 2000 years.

The Little Ice Age. One of the more well-documented major Holocene cooling events, which is also recent, is the Little Ice Age (LIA). The LIA is defined as the period of cooling after the medieval warm period (~1300 A.D.), lasting until the mid-19th century (Petersen, 1994; Luckman, 2000). The latest phase of glaciation in the Beartooth Mountains documented by Graf (1971) is likely from the LIA. Similar LIA glacial advances have been documented in the Front Range of Colorado 300-100 years Before Present (BP) (Benedict, 1973) and in the Wind River Range of western Wyoming, with a maximum extent likely during the mid-19th century (Meier, 1951). Graf (1971) postulates that the Pinedale age glacial features in the Beartooth Mountains, are time synchronous with similar features in the Wind River Range, and it seems reasonable to assume that the glacial features formed in the LIA in the Beartooth Mountains, Wind River Range, and Colorado Front Range are all a response to broadly similar regional climatic forcing.

Study Site

Two catchments (a northern and southern catchment, hereafter referred to as the Forget-Me-Not and Albino Catchments respectively, see Figure 2.1 in Methods) were examined that have similar elevations, bedrock geology, area, lakes, and atmospheric inputs. The major difference in the catchments is the presence of a glacier in the northern Forget-Me-Not Catchment, and the absence of any perennial ice features in the southern Albino Catchment. This paired catchment approach compares nitrogen dynamics in glacial and unglaciated systems. The paired catchments investigated in this study are the catchment that drains Forget-Me-Not Glacier to the southwest, draining into Jasper Lake, and the catchment directly south, lacking any perennial ice features, which contains Albino Lake (Figure 2.3). For an overview figure showing all sites located within the two catchments see Figure 2.4. The elevation range from Albino Lake to the top of the Forget-Me-Not Drainage spans from 3050m-3725m respectively (Figures 2.5, 2.6, 2.7, and 2.8).

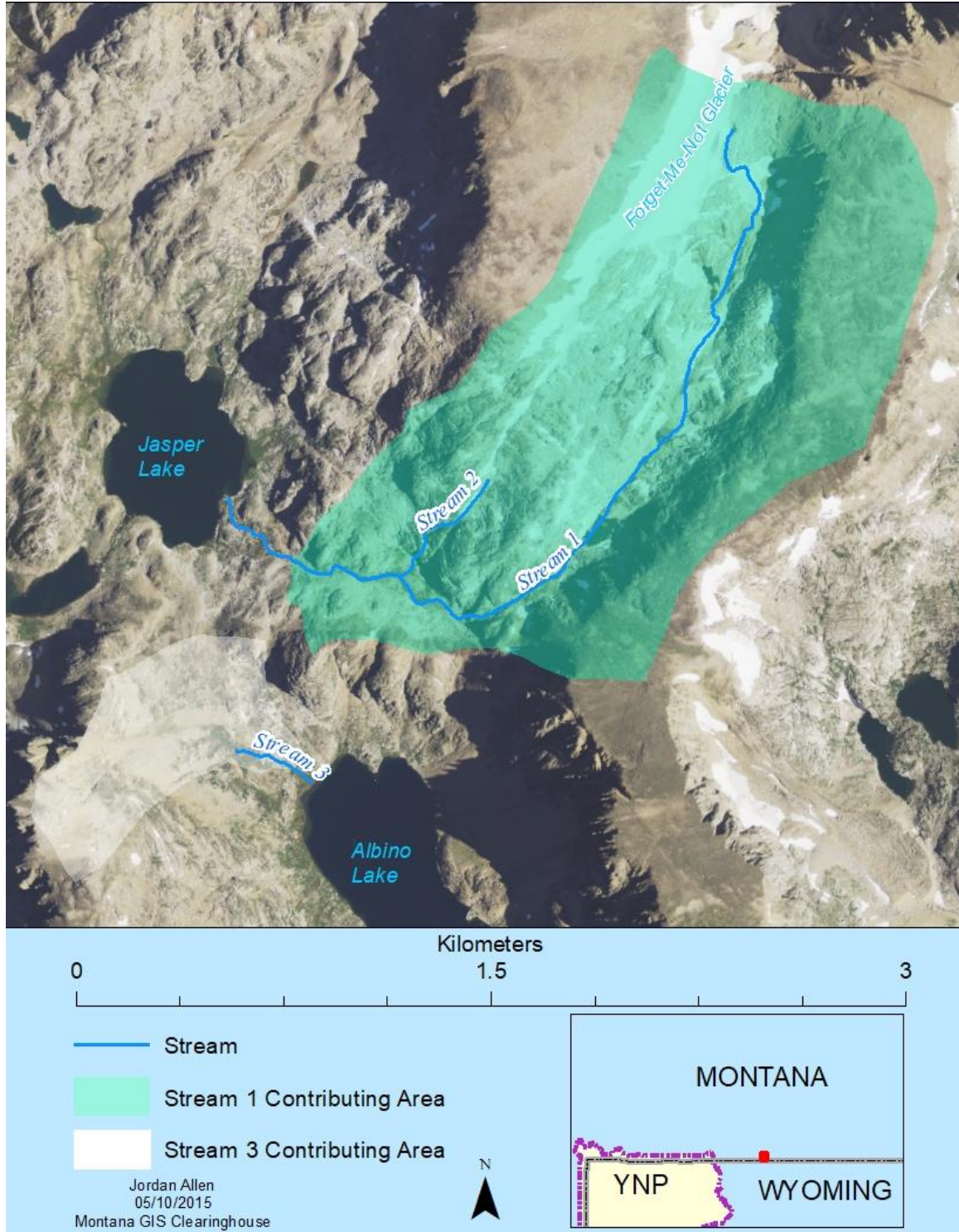


Figure 2.3. Field area location just north of the Wyoming-Montana border. The two catchments are shown with contributing areas shaded. The Jasper Catchment is fed in part by the Forget-Me-Not Glacier, while the Albino Catchment is not fed by any perennial ice features.

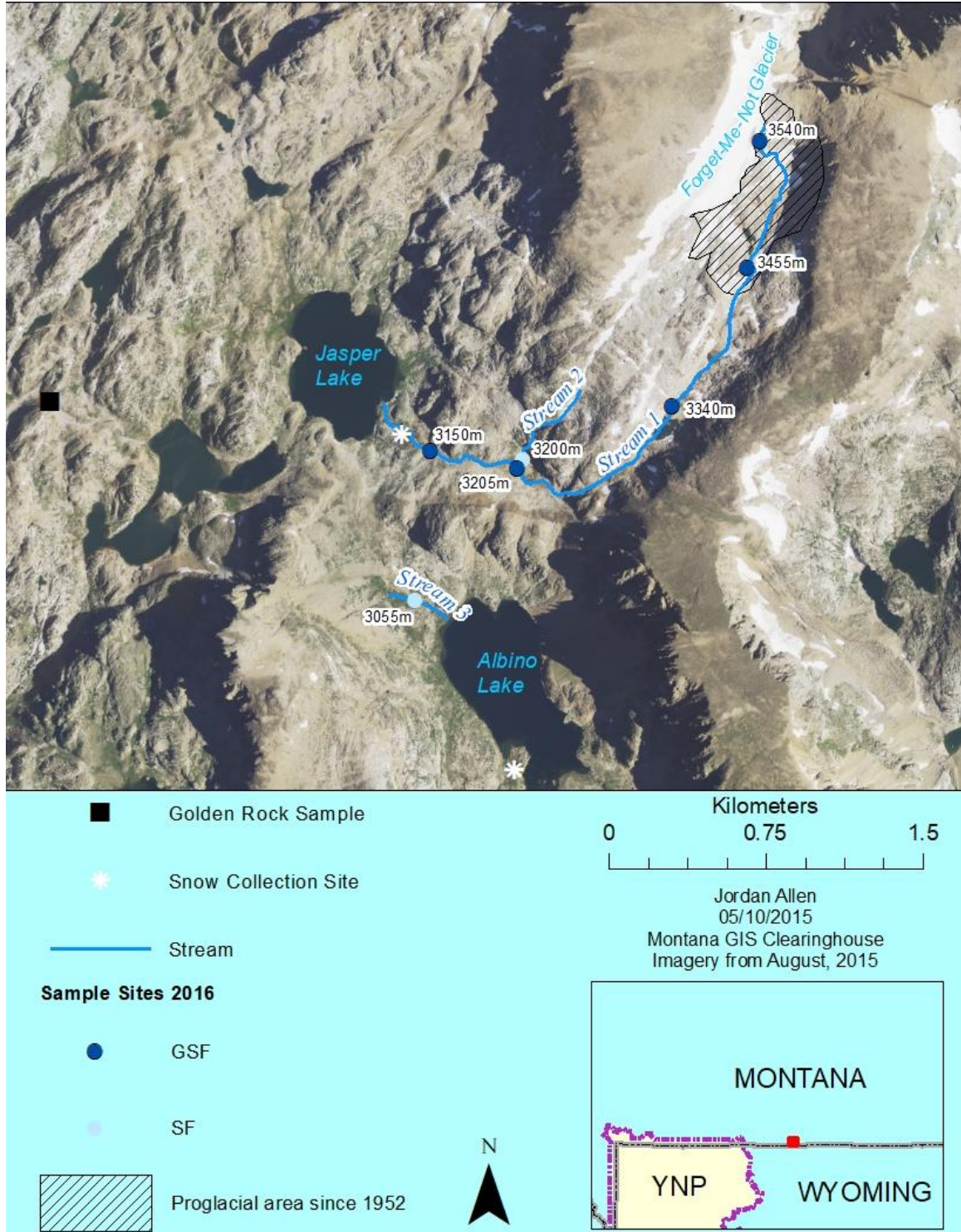


Figure 21.4. Overview figure showing sample sites of stream water for 2016, summer snow sample sites, and the rock sample collected northwest of Golden Lake (three other rock samples were collected in the proglacial area, shown on this map in the cross-hatched region directly southeast of Forget-Me-Not Glacier).

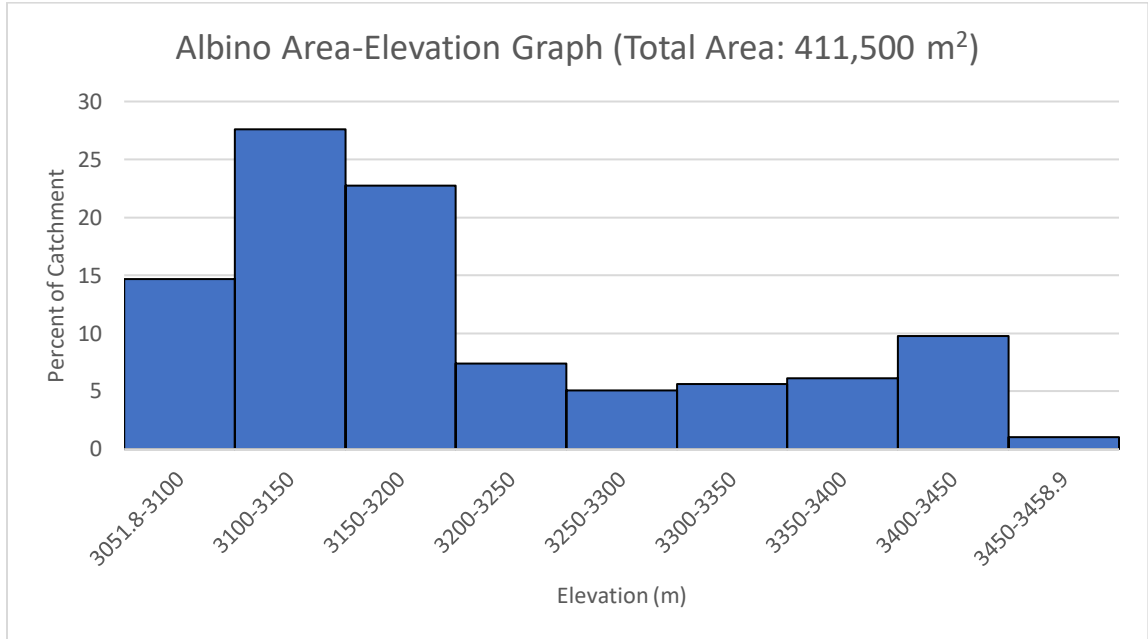


Figure 2.5. Albino Catchment area-elevation graph showing the percent of total catchment area in 50m elevation segments from the bottom of the catchment to the top. It should be noted that the lowest and highest bins are not 50m.

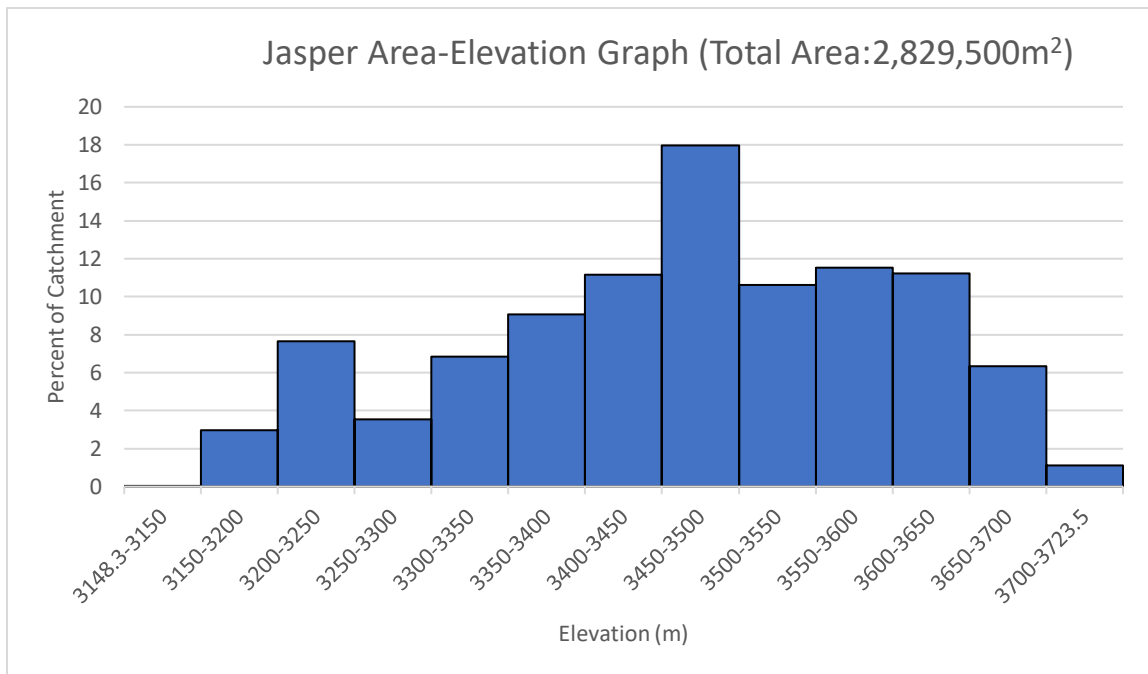


Figure 2.6. Jasper Catchment area-elevation graph showing the percent of total catchment area in 50m elevation segments from the bottom of the catchment to the top. It should be noted that the lowest and highest bins are not 50m.

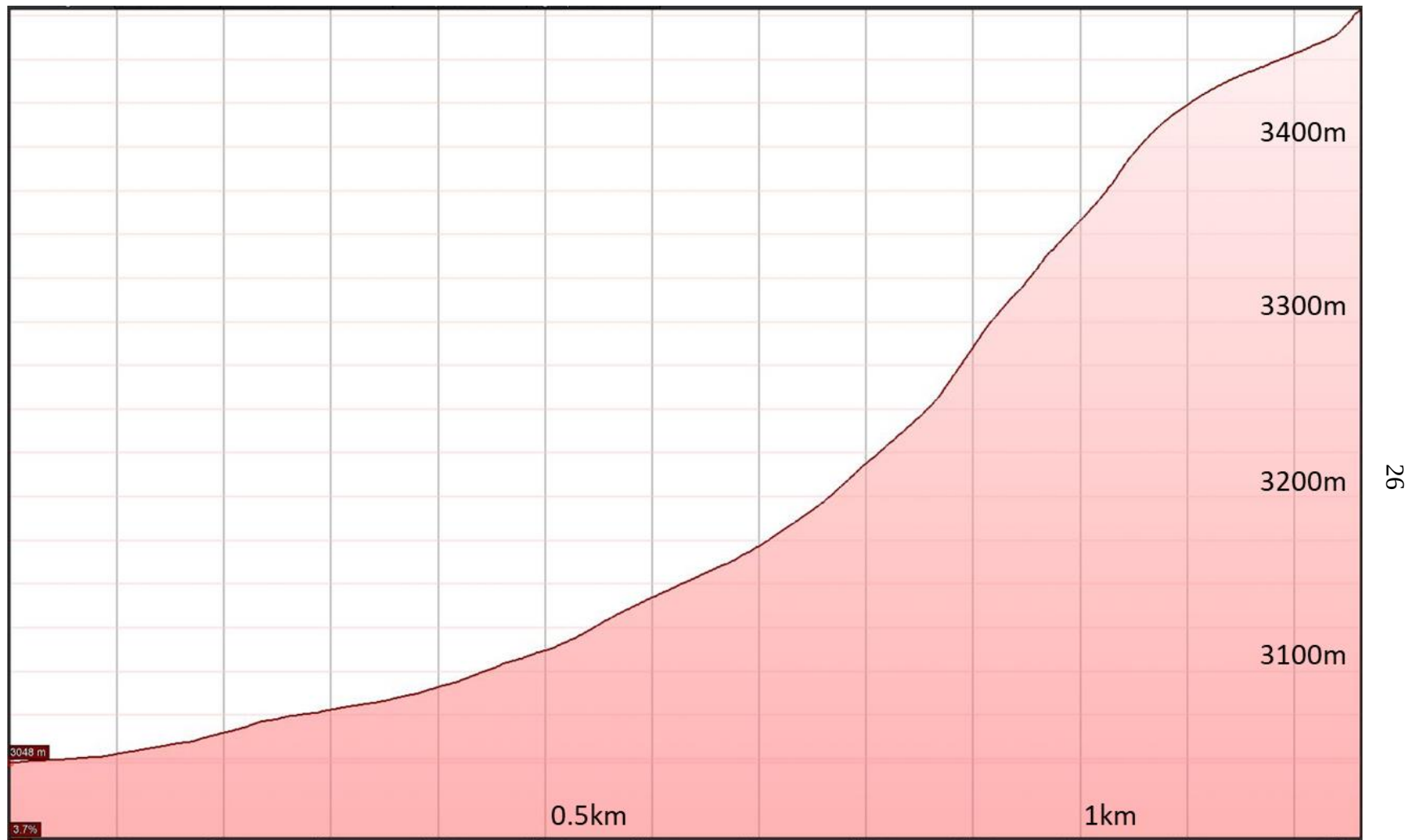


Figure 2.7. A longitudinal profile up the Albino Catchment, following the stream to its source, then following slope to the top of the catchment. Note slightly different elevation and horizontal ranges (scales) between the two catchments.

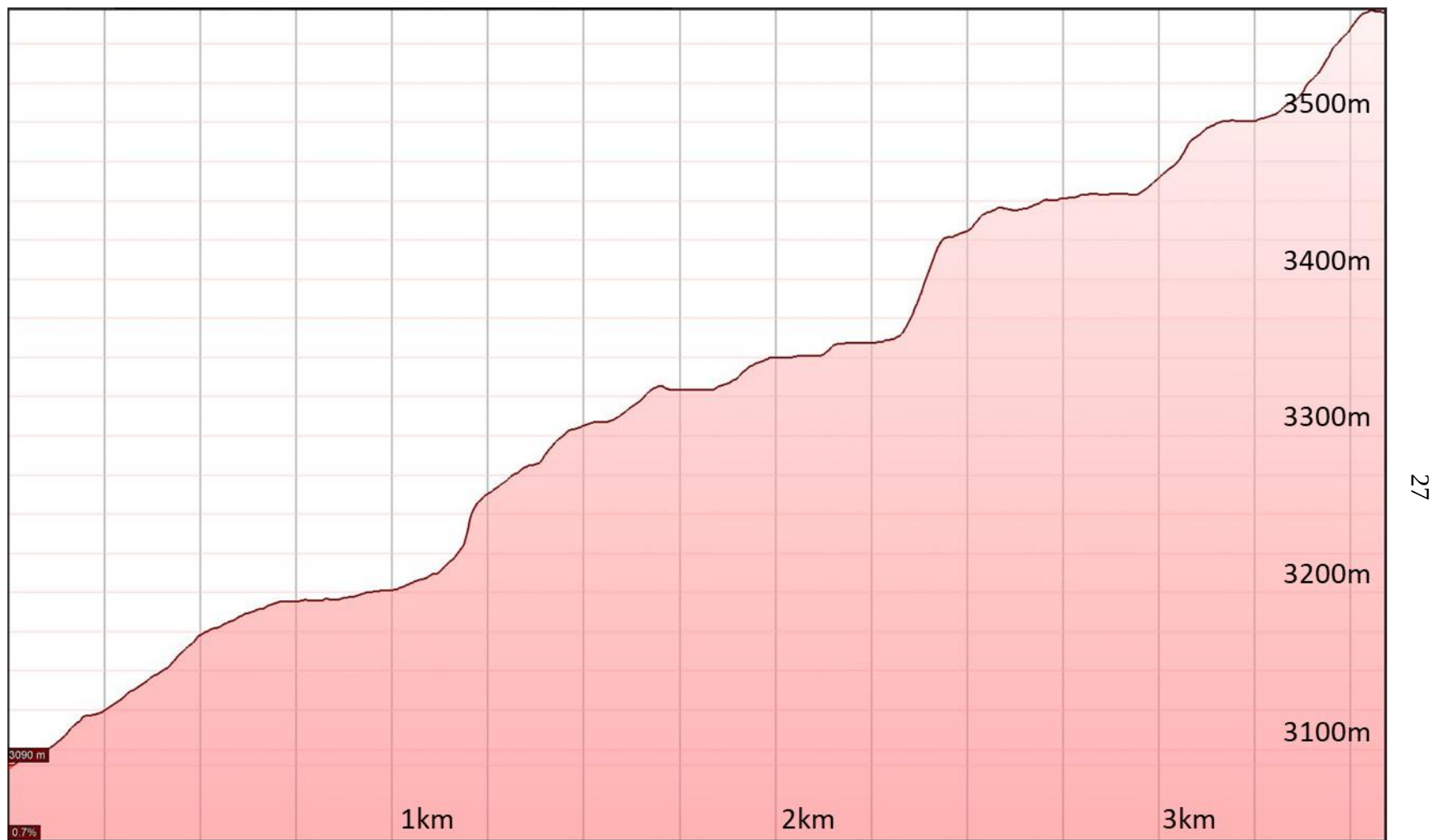


Figure 2.8. A longitudinal profile Jasper Catchment, following the stream to its source, then following slope to the top of the catchment. Note slightly different elevation and horizontal ranges (scales) between the two catchments.

Significant perennial streams exist in the field area, and the physical, chemical and microbiological characteristics of three of these streams were examined in this research (Figure 2.3). Stream 1 drains from Forget-Me-Not Glacier, eventually flowing into Jasper Lake. A tributary of this stream, stream 2, that does not originate at the glacier was also examined. The largest stream in the southern catchment, stream 3, which drains into Albino Lake, is used as a control, as it has no recent glacial source.

Forget-Me-Not Glacier, in its current configuration, flows from the NW to the SE. The glacier appears to receive extra deposition, relative to surrounding areas, derived from a large high plateau to the west of its location, and the break in slope where the glacier begins (Figure 2.4). The prevailing west winds in the region, appear to cause sufficient wind-loading of snow on the glacier to either negate or slow its retreat in its current configuration. Though it is much wider than it is long, it is unlike a typical cirque glacier in that it is not surrounded by steep walls that could protect it from solar insolation or provide topography that could produce avalanching of snow and/or debris onto the glacier.

Field Measurements and Sample Collection

Snow Sampling

Snow was collected from various places in and adjacent to the two catchments at various times throughout the study (see Figure 2.9). Snow was collected as close to peak snowpack as possible during the spring of both 2015 and 2016. Due to the nature of the field area (Wilderness designation) sampling snow from the actual catchments was not

possible in the winter months. Therefore, the spring sampling locations were chosen as close to the catchments as practical for sampling during peak snowpack. Exact locations for sampling were chosen based on their distance from large vegetation (willows and trees) and their distance from visible human activity (the area is frequented by motorized recreationalists).

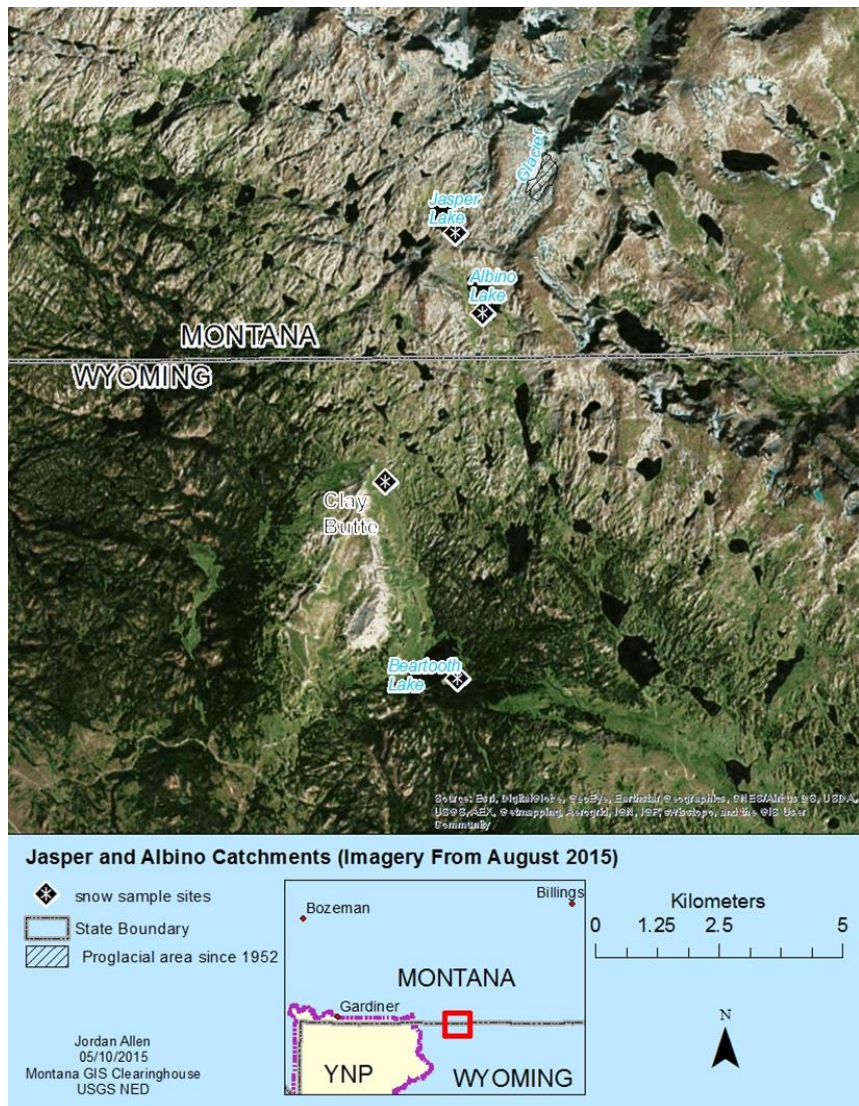


Figure 2.9. Snow sampling locations. Samples from Beartooth Lake were taken on May 19, 2015, from north-east of Clay Butte on April 9, 2016, from Albino Lake on September 1, 2014 and October 1, 2014, and from the southeastern bank of Jasper Lake on October 1, 2016.

Snow was sampled using sterile techniques, cleaning the metal scoop (shovel or dustpan) between samples with alcohol and rinsing in adjacent snow from the same layer as was sampled.

Samples from Beartooth Lake were taken on May 19, 2015 (Figure 2.9 and Figure 2.10). The snow pit dug was ~90cm deep. Snow was sampled in large Whirlpak bags at heights of 25cm, 40cm, and 90cm (the surface) from the base of the snow pack. Four medium Whirlpak bags each were filled with snow at 60cm and at the surface. Triplicate samples were taken sterilely at 20-25cm, 40-45cm, and 86-90cm in 50ml polypropylene conical tubes. Density measurements were also taken of the snow in duplicate for 0-30cm, 30-60cm, and 60-90cm. Snow temperature was measured at two depths in the snowpack profile and the snowpack was isothermal at 0°C. Air temperature was also measured at 7.5 °C (Table 2.1).

Table 2.1. Beartooth Lake snowpack samples, May 19, 2015.

Distance to ground (cm)	Average Density (g/liter)	Temperature °C	Sterilely sampled Whirlpak	Rinsed Whirlpak samples
Surface	NA	air 7.5	1	4
60-90	452	0	NA	4
30-60	401	0	1	NA
0-30	478	NA	1	NA
Ground	NA	NA	NA	NA



Figure 2.10. Sampling of isolated layers in the snowpack at Beartooth Lake, May 19, 2015. This was close to the peak snowpack for the 2014-2015 snow year.

Samples were taken April 9, 2016 north-east of Clay Butte (Figure 2.9 and Figure 2.11). The snow pit was ~131cm deep (Table 2.2). The bottom 10cm was sampled using one large Whirlpak bag. The bottom 10cm of snow was sampled separately from the other snow due to debris from the ground contaminating this lowest portion of snow. The top ~120cm was divided into four 30cm increments: 10-40cm, 40-70cm, 70-100cm, and 100-130cm. Each of these 30cm increments had four large Whirlpak bags of snow collected. Two 50ml samples were also taken from each of the four 30 cm increments. Snow was sampled sterilely. A profile of snow depth, temperature, grain form, grain size, and hardness was taken and is shown in Figure 3.1 in the Results.

Table 2.2. Clay Butte snowpack samples April 9, 2016.

Distance to Ground (cm)	Temperature	50ml Sterile Samples	Large Whirlpaks
Surface	5	NA	NA
131-100	NA	2	1
70-100	NA	2	1
40-70	NA	2	1
10-40	NA	2	1
0-10	NA	NA	1



Figure 2.11. Snow sampling 0.5km northeast of Clay Butte, April 9, 2016. This was close to the peak snowpack for the 2015-2016 snow year.

Snowfall was collected during the summer months in 2016 and 2014.

Approximately 500g of snow was sampled using sterile tools, on the southeastern bank of Jasper Lake on October 1, 2016 (Figure 2.9). Snow (~1kg) was collected by others (Dr. Mark Skidmore and Will van Gelder) along the shore of Albino Lake September 1, 2014. Fresh snow was sampled sterilely.

Stream Sampling

Sampling Sites. Samples of water, stream sediment and suspended sediments, and cells were obtained at seven different sites throughout the two catchments during the 2016 summer field season (see Figure 2.12, Table 2.3). The Albino Catchment sample location (3055m, non-glaciated) was chosen because this was the lowest, non-distributary portion of the major Albino Lake inlet stream. Five locations were chosen for the Jasper Catchment (glaciated) as follows. They are listed below in descending order from the glacier. Site 3540m was chosen as it was a major outflow of subglacial water that could be consistently sampled throughout the field season. Site 3455m was selected because it is at the approximate extent of the glacial coverage in 1952 (the oldest available imagery). This selection ensures that all of the catchment directly above this site was recently glaciated. Site 3340m was chosen as a midway location (between the proglacial area and other non-glaciated parts of the overall catchment). Site 3200m is just upstream (~50m) from the confluence of the non-glaciated stream (stream 2) and the main glacially-sourced stream (stream 1); and is the lowest portion of the glaciated stream with no other major inputs other than the glaciated portion of the catchment. Site 3150m is the

lowest non-distributary portion of the major Jasper Inlet stream (stream 1). Site 3205m non-glacial (ng) was a tributary (stream 2) of the main glacial stream and is not fed by a currently glaciated area.

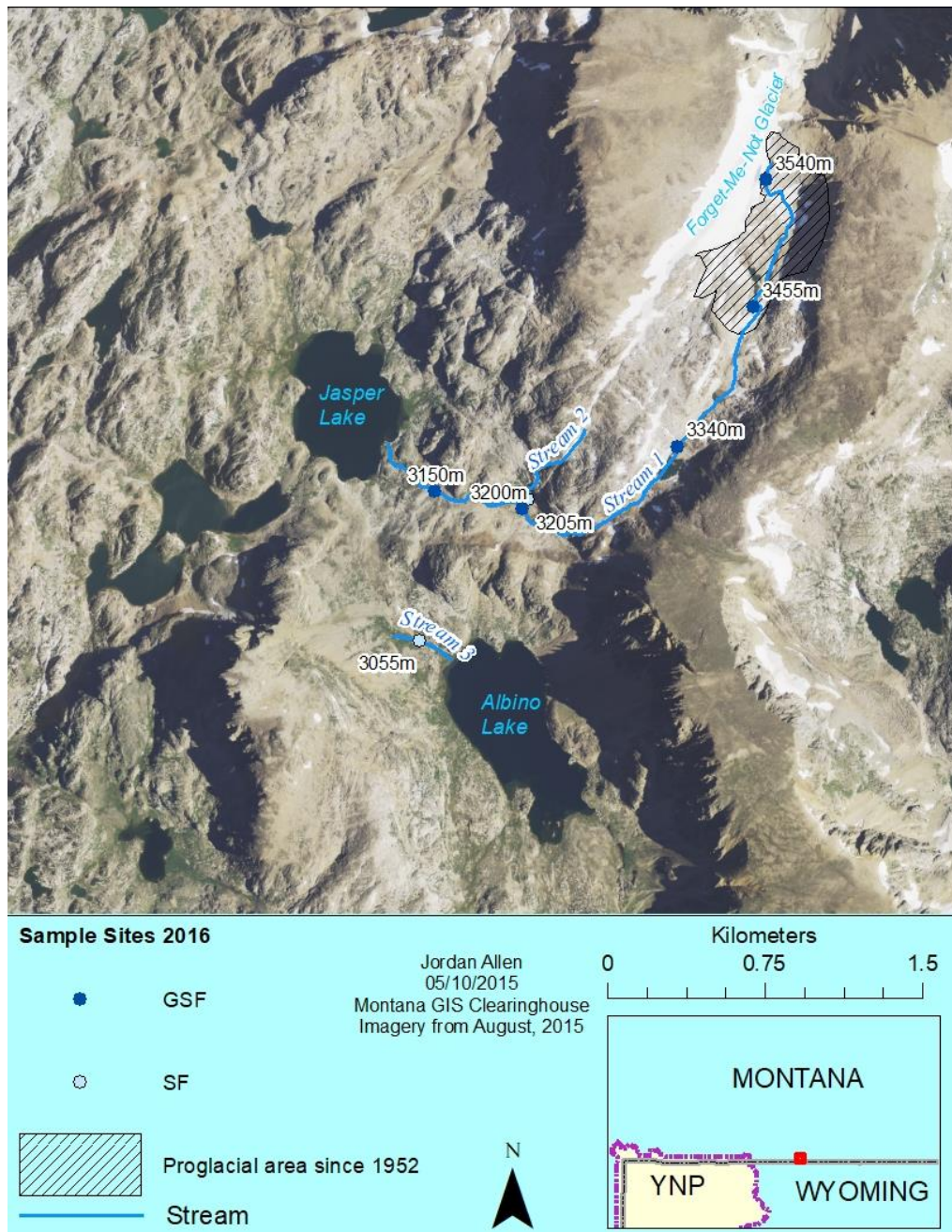


Figure 2.12. Locations of sampling sites for water, sediment, and suspended sediment and microbial cells during the 2016 season.

Table 2.3. Timetable showing samples collected each field sampling visit and field measurements. Water measurements, filtered samples for analysis of anion and cation concentrations, pH, EC, and temperature were taken at each of the sample sites except for the highest elevation sites on day of year (DoY) 189-190.

	2014	2015	2016	Day	Day	Day	Day	Day	Day	Day
Sample Collection	Sept	May	Apr	189-190	200-201	214-215	228-229	238-239	252-253	274-275
Water Measurement										
Samples				x	x	x	x	x	x	x
pH				x	x	x	x	x	x	x
EC				x	x	x	x	x	x	x
Temperature				x	x	x	x	x	x	x
Discharge salt							x	x	x	x
Sterivex										x
Sediment				x						
Rock									x	
Snow	x	x	x		x					

Water Sampling. Water samples were collected in the two catchments over the summer months during the 2016 field season at the sites highlighted above (Table 2.3). Water samples taken during the summer 2016 field season from July 6 through October 1, 2016 (DoY 189-275) as follows: one water sample (500ml) per site was collected and vacuum filtered through a 0.4µm Nuclepore filter (Table 2.3). On October 1, 2016 (DoY 275), the vacuum pump broke, so water samples (250ml) were filtered using 0.45µm Sterivex filters. Samples were collected at each site on each sampling date of the 2016 field season with the exception of 3340m, 3455m, and 3540m which were unreachable (due to snow cover) until July 18, 2016 (DoY 201). In both field seasons filtered samples were placed on snow while field work continued, placed in frozen storage for transport, and frozen upon return from the field.

Sediment Sampling. Stream sediments were sampled at site 3055m, 3150m, 3200m, and 3205m (non-glacial tributary) during the 2016 summer field season. Sediment was sampled using sterile scoops and 500ml bottles acid washed (3X) and rinsed (3X) in adjacent stream water (~40ml per site) on July 7, 2016 (DoY 190) (Table 2.3). Samples were placed on snow while field work continued, placed in a frozen storage for transport, and frozen upon return from the field.

Biomass Sampling. Each of the stream sites was sampled on DoY 274-275 in 2016 for biomass, by pushing 1L of stream water through 0.45 μ m Sterivex filters using a sterile, 150ml, syringe, rinsed (3X) in sampled stream water. The filters were stored in 50ml polypropylene conical tubes and placed on snow while field work continued, placed in a frozen storage for transport, and frozen upon return from the field.

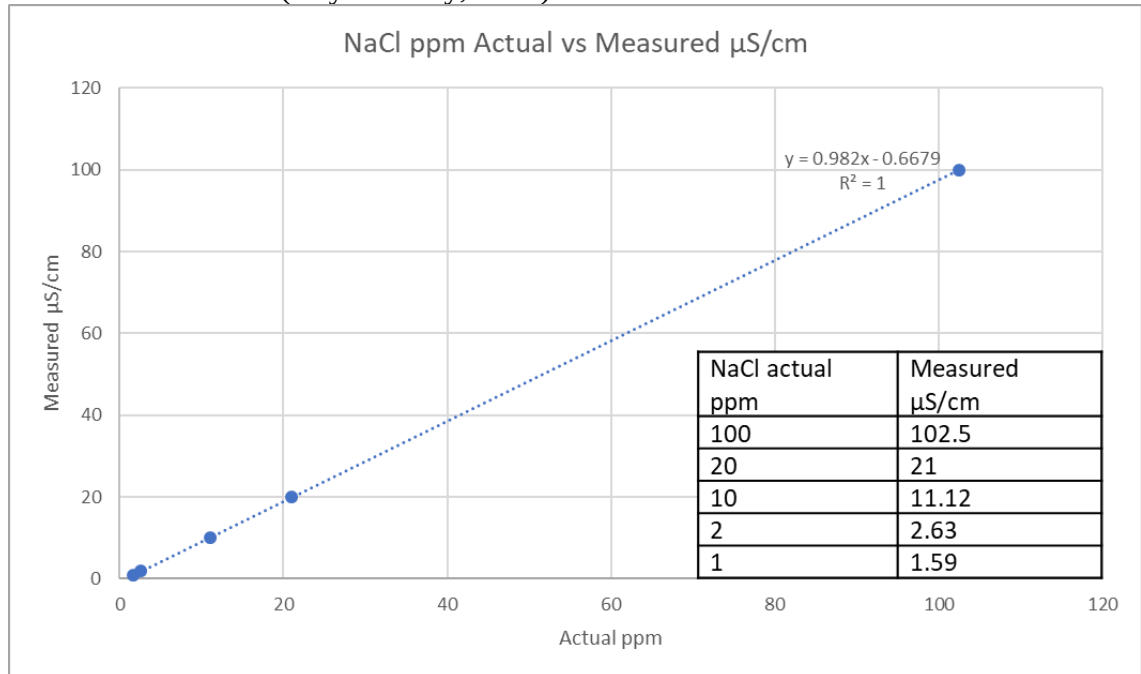
Water Measurements. At each sample site stream water temperature, electrical conductivity, and pH (Table 2.3) were measured using a Mettler Toledo Probe. Temperature measurements were within 0.1°C at both 4°C and 20°C. Electrical conductivity readings were found to be within 0.1 μ S/cm at 20°C for standards at 5, 10, and 20 μ S/cm.

Stream Discharge Measurements. Discharge was measured at the time of water sampling during the 2016 field season immediately below the confluence of the two streams measured at 3205m and 3200m. Discharge was measured at this site, because it

was the lowest site (closest to site 3150m) where there was a straight enough 30m long reach to perform discharge measurements. Cross-sectional measurements of stream depth were taken at each time point at 10cm intervals, with the stream width ranging from 2.6m to 3.9m depending on discharge.

A salt-slug method was used as a measurement to estimate discharge for the time points from August 16, 2016 through October 1, 2016 (DoY 229-275). A 1L 20%NaCl solution was used on August 16, 2016 and August 25, 2016, and a 1L 10% NaCl solution was used September 9, 2016 and September 30, 2016. The salt solution was added to the top of the 30m reach and electrical conductivity measurements were taken in the stream every 5s as the slug approached, peaked, and returned to background levels with total measurement times ranging from 265-540s. Calibration of the electrical conductivity probe was performed with known concentrations of NaCl in order to determine the relationship between salt concentration and electrical conductivity, results of the calibration are shown in Figure 2.11. Error in discharge measurements using this method are typically around 5% (Day, 1976).

Figure 2.13. Salt calibration which yields a nearly 1:1 relationship between conductivity in $\mu\text{S}/\text{cm}$ and NaCl concentration in ppm. A direct 1:1 relationship was used as is convention (Day and Day, 1977).



Areas under the conductivity curves (trapezoidal method, using the 5 second intervals) were used to determine the concentration of salt, which can be used as a proxy for stream discharge using the Salt Discharge Equation seen below:

$$\text{Salt Discharge Equation: } Q = \frac{(C_T - C_b) * V_T}{\sum_0^{\infty} [C_d(t) - C_b] * dt} \quad (\text{Equation 4})$$

where C_T is the concentration of the tracer solution, C_b is the background concentration of the tracer in the stream, V_T is the volume of the tracer solution added. $C_d(t)$ is recorded as a function of time until it recedes to the original background level (C_b) (Figure 2.14) (Dingman, 2015). The conductivity curves (Figure 2.14) are typical for salt slug measurements, showing a sharp increase in conductivity as the slug arrives, with a gentler recession limb back to base-level. Photos of the discharge measurement site and surrounding vicinity are shown in Figures 2.15, 2.16, and 2.16.

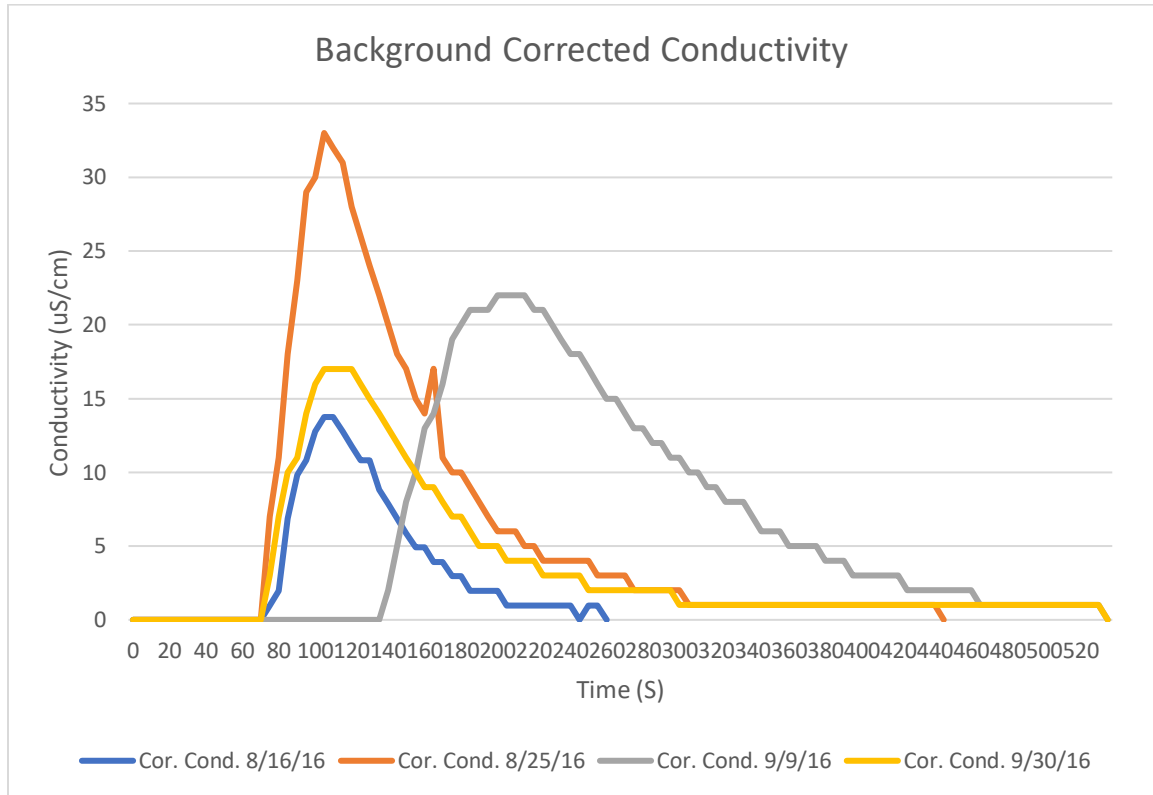


Figure 2.14. Stream conductivity response following salt slug addition to the stream.



Figure 2.15. Location a) of discharge measurements (looking due east), where the cross section of the stream was taken. Note the large cascade in the upper-right of the photo, which also had another cascade splitting the flow of the main glacial waters out of view (Figure 2.16). The non-glacial component of the stream can also be seen in the photo in the upper left; this component was much smaller and is only visible as wetted rock in this photo, though a small stream did form as the water coalesced at the bottom of the rock wall. This photo was taken on July 7, 2016, DoY 189. b) The cascade in the background is markedly smaller. The stream, while at a slightly different angle (still near due east), can be seen as markedly smaller than on the first outing. This photo was taken on September 30, 2016, DoY, 274.



Figure 2.16. This is the same location as Figure 2.15 but looking due south, perpendicular to Figure 2.13. This photo was taken on August 25, 2016, DoY 238 after a significant snowfall (~7cm) the night prior. Here a field technician is performing measurements taking the cross-sectional area of the wetted perimeter.



Figure 2.17. Showing a closer view of the cascade visible in Figure 2.13. The right half of the cascade is not visible in Figure 2.13a and was not flowing on Figure 2.13b. This photo was taken on DoY 200 (July 17, 2016) when discharge values were at their highest.

Rock Sampling

Rocks (~2kg) were sampled during both the 2015 and 2016 field seasons. A detached rock that was representative of the two catchments based on major minerals was collected near Golden Lake, just west of Jasper Lake in 2015 (Figure 2.2). Three rocks were also collected from the proglacial area of Forget-Me-Not Glacier in 2016 (Figure 2.4). Two of these rocks were chosen because they were most representative of the catchment overall based on observation of major minerals (quartz, feldspars, and amphiboles). The other was chosen for its unique lithology compared to the majority of

the upper catchment. Thin sections were made from each sample as detailed in the “Laboratory Methods” section.

Laboratory Methods

Abiotic Rock Weathering

Rocks collected (~2kg per sample) during the 2015 and 2016 summer field season were cut using a rock saw such that none of the previously exposed and/or oxidized surfaces were part of the sample that was further processed. Unoxidized surfaces yielded samples of ~200g per sample. Glass vials (20ml) were prepared as follows: deionized water (DIW) rinsing (three times) followed by five hours in a furnace at 500°C. Septa caps were autoclaved at 123°C for 30 minutes. The rock samples were heated at 105°C for one hour to ensure sterility and then crushed using a Braun Chipmunk Jaw Crusher model VD67, followed by further grain size reduction using a Bico Pulverizer type UA into pieces 2cm³ or smaller in size suitable for introduction into the grinding mill, equipped with a tungsten carbide bowl and ball bearings (Fritsch mono mill PULVERISETTE 6). The crushed rock fragments were milled for one hour, resulting in a homogenous, very fine (average grain size of 3µm) powder. The jaw crusher and mill were cleaned between samples by crushing high purity quartz. The quartz residue was then cleaned away using compressed air and vacuuming up the loose material with a Shop-Vac vacuum cleaner. The crushing surfaces were then wiped down with ethanol.

Slurries of DIW and the freshly crushed rocks were created by adding ~7g of crushed rock and 10ml of 0.2µm filtered DIW to the glass vials. The vials were capped

with the septa, crimp sealed, and were placed on a shaker at 150 rpm at 4°C in the dark. Experiments were performed in triplicate. Controls of DIW were performed using the same vials, caps, and filtration. The reactions were terminated after 5 minutes (T_0), 69 hours (T_2), and 330 (T_3) hours via filtering the slurry using 0.1 μ m syringe filters. The filtrate was stored frozen at -20°C. Single samples of water were later thawed and then analyzed for major anion (F^- , Cl^- , NO_2^- , Br^- , NO_3^- , PO_4^{3-} , SO_4^{2-}) and cation (Li^+ , Na^+ , NH_4^+ , K^+ , Ca^{2+} , Mg^{2+}) concentrations, including dissolved inorganic nitrogen species, ammonium and nitrate using an ion chromatograph, to determine ion release from the milled rocks during the exposure to the DIW.

Ammonium and Nitrite Oxidizer Enrichments

Ammonium and nitrite oxidizer specific media (Atlas, 2010; Enrich et al., 1995) were prepared in the laboratory and inoculated with glacial sediments in order to enrich for ammonium oxidizing and nitrite oxidizing organisms (see Appendix Oxidizer Media for recipes). Approximately 2g of sediment was added to 10ml of pre-chilled, ammonia-oxidizer media (Atlas, 2010) or nitrite-oxidizer media (Enrich et al., 1995) from sites: 3055m (non-glacial), 3150m, 3200m, and 3205m (non-glacial). The enrichments were all prepared in triplicate along with two control sets also in triplicate, uninoculated media and DIW. Samples were placed on a shaker at 150 rpm at 4°C in the dark for one month. After a month of incubation, samples were vortex mixed before 1ml of the initial slurry/media was added to 9ml of new enrichment media in 20ml septa capped vials, ensuring the transfer of some of the original sediment and replaced on the cold-room

shaker. This transfer process was repeated after an additional month of incubation. The original enrichment began on July 13, 2016 (T_0) and transfers were performed on day 32 and day 62. Samples (5-8ml) were taken on day 97 and filtered using 0.4 μ m syringe filters and frozen for later analysis of anion and cation concentrations via ion chromatography. Standards were run in parallel with the samples for major anions at concentrations of 0.01, 0.05, 0.1, 0.5, 1, 10, and 20ppm and major cations at concentrations of 0.01, 0.05, 0.1, 1, 10, and 20ppm.

XRF

XRF analysis of the representative rock obtained in the 2015 summer field season was performed by the Washington State University GeoAnalytical Lab (<https://environment.wsu.edu/facilities/geoanalytical-lab/>) to determine elemental composition of the rock. XRF analysis was completed following Johnson et al., (1999).

Major Anion and Cation Analyses

Stream water samples collected at each site for each sampling period (Table 2.3) were analyzed on a Metrohm Peak Ion Chromatograph for major anion (F^- , Cl^- , NO_2^- , Br^- , NO_3^- , PO_4^{3-} , SO_4^{2-}) and cation (Li^+ , Na^+ , NH_4^+ , K^+ , Ca^{2+} , Mg^{2+}) concentrations, including dissolved inorganic nitrogen species, ammonium and nitrate. Frozen samples from the field were melted and analyzed with known standards for anions at concentrations of 0.1, 0.5, 1, 5, 10, and 20 ppm, and for cations at concentrations of 0.01, 0.05, 0.1, 0.5, 1, 5, 10, and 20 ppm. Blanks (18.2 M Ω DIW) were run as a control. Anion

concentrations varied less than 0.1ppm and linear regression of known cation

concentrations and outputs showed R^2 values of 0.999 or greater.

Cation concentrations varied less than 0.1ppm and linear regression of known cation

concentrations and outputs showed R^2 values of 0.998 or greater.

DNA Extraction and 16S rRNA Gene Sequencing

Genomic DNA was extracted from the Sterivex filters collected from sites 3150m, 3340m, 3455m, 3540m, 3205m (NG), and 3055m (NG). DNA extraction and purification were performed using Fast DNA Spin Kit (<https://www.mpbio.com/>) following Boyd et al., (2007). Qualitative and quantitative PCR analysis were performed on the purified genomic DNA using bacterial primers (1100F-1492R) to confirm the presence of bacterial 16S rRNA genes (Turner et al., 1999). Samples of purified DNA were sent to MR DNA for analysis of bacterial diversity (<http://www.mrdnalab.com/>) using bacterial 16S primers (1100F-1492R) (Turner et al., 1999). Amplicons from each sample were barcoded as outlined by Dowd et al., (2008). Post sequence processing was performed using Mothur software (Schloss et al., 2009) as described by Hamilton et al., (2013).

Stable Isotopic Analysis of Nitrate in Snow and Stream Water

Analysis of $\delta^{15}\text{N}$, $\delta^{18}\text{O}$, and $\delta^{17}\text{O}$ of the nitrate was performed on samples taken from the field from various sources and times: glacial and non-glacial stream waters from the 2016 field season (from streams 1, 2, and 3), bulk snowpack from the 2014-2015 and 2015-2016 snow year, and fresh summer precipitation (in the form of snow) from the

2014 and 2016 summer field seasons (see Table 2.4). Glacially sourced water taken from stream 1 at the earliest dates where all sites were visited (day 200 and 201 of the 2016 season) were used to get the earliest signal of isotope values at the start of the melt-season. Similarly, glacial stream water was taken as nitrate levels showed a marked increase (days 238 and 239 of the 2016 melt-season) to see if there was an isotopic change that could be seen with the concentration change (Figure 3.3). Waters from the glacial stream were also analyzed at the end of the 2016 melt-season (days 274 and 275) to give the widest span of time on isotopic data. Waters from stream 2 were also analyzed at the same time periods, as it mirrored stream 1 chemistry (Figure 3.3). Some of stream water and atmospheric input had very low nitrate concentrations and required concentration before isotopic analysis could be performed (Table 2.5).

Table 2.4. Samples analyzed for $\delta^{15}\text{N}$, $\delta^{17}\text{O}$, and $\delta^{18}\text{O}$, with nitrate concentrations. Samples with concentrations below $5\mu\text{M}$ were concentrated into a final volume of 10ml via ion exchange chromatography. Nitrate concentrations are within $0.1\mu\text{M}$.

Site	Day	Date Collected	Water Type	Fresh Snow	Nitrate Concentrations μM	Concentrated	Amount Concentrated (ml)
3055m	275	10/1/2016	Stream 3	NA	1.3	Yes	150
Blank DIW	NA	NA	DIW	NA	BDL	Yes	150
Beartooth Lake	NA	5/19/2015	Snow	Yes	1.6	Yes	100
Albino Lake	NA	9/1/2014	Snow	Yes	11.6	No	NA
Jasper Lake	200	7/18/2016	Snow	No	26.9	No	NA
3150m	200	7/18/2016	Stream 1	NA	35.2	No	NA
3150m	228	8/15/2016	Stream 1	NA	37.1	No	NA
3150m	274	9/30/2016	Stream 1	NA	112.2	No	NA
3200m	200	7/18/2016	Stream 1	NA	31.4	No	NA
3200m	229	8/16/2016	Stream 1	NA	72.4	No	NA
3200m	274	9/30/2016	Stream 1	NA	120.7	No	NA
3205m	200	7/18/2016	Stream 2	NA	25.9	No	NA
3205m	229	8/16/2016	Stream 2	NA	83.2	No	NA
3205m	274	9/30/2016	Stream 2	NA	109.9	No	NA
3340m	201	7/19/2016	Stream 1	NA	20.2	No	NA
3340m	229	8/16/2016	Stream1	NA	70.8	No	NA
3340m	275	10/1/2016	Stream 1	NA	95	No	NA
3455m	201	7/19/2017	Stream 1	NA	17.1	No	NA
3455m	229	8/16/2016	Stream 1	NA	47.3	No	NA
3455m	275	10/1/2016	Stream 1	NA	64	No	NA

Nitrate was concentrated by adding a volume (see Table 2.4) of filtered sample to Bio-Rad Poly-Prep Chromatography Columns using AG 1-X8 resin, and 200-400 mesh, following Frey et al., (2009). Sample amounts added to the columns varied according to the concentration of nitrate present in the initial sample (Table 2.4). Concentrated nitrate was eluted from the columns using, high purity (99.998% Alfa Aesar Puratronic), 1M NaCl. $\delta^{15}\text{N}$, $\delta^{18}\text{O}$, and $\delta^{17}\text{O}$ determinations were undertaken at the University of Washington IsoLab (<http://isolab.ess.washington.edu/isolab/sample-prep-analysis/denitrifier>) using the bacterial denitrifier method, a Gas Chromatograph to separate pyrolyzed N_2 and O_2 , and then the gases are introduced into the isotope ratio mass spectrometer (Casciotti et al., 2002; Kaiser et al., 2007; Sigman et al., 2001).

Accuracy and precision of stable isotope determinations can be seen in Table 2.5.

Table 2.5 Accuracy and Precision based on International Atomic Energy Agency (IAEA) NO_3^- for nitrogen and IAEA NO_3^- for oxygen.

	Accuracy	Precision	Units
$\delta^{17}\text{O}$	0.07908	0.39261	permil
$\delta^{18}\text{O}$	-0.26906	0.15906	permil
$\Delta^{17}\text{O}$	0.21899	0.36006	permil
$\delta^{15}\text{N}$	-0.0354	0.087185	permil

Thin Sections

Thin sections of the one rock collected in 2015 and the three collected from the proglacial area in 2016 (see the “Rock Sampling” section of the Methods) were processed by Wagner Petrographic using standard procedures after being cut to billets at MSU. Final thin sections were 24 x 46mm and ~30 microns in thickness; slides were also impregnated with clear epoxy (<http://www.wagnerpetrographic.com>). Slides were

analyzed on a Nikon petrographic microscope for mineralogy and likely rock histories (e.g. the presence of metasomatic conditions).

Modelling of Air Masses Supplying Precipitation to the Catchments

Atmospheric deposition of inorganic N could originate from multiple sources, anthropogenic or natural (Holloway et al., 1998). Analyzing potential air mass movements prior to precipitation events in the study area was performed to constrain potential sources of inorganic N deposited during precipitation events. Analysis of air mass movement for the five days prior to precipitation events was performed using Hybrid Single Particle Lagrangian Integrated Trajectory (HYSPLIT) model, which is a resource from the National Oceanic and Atmospheric Administration (NOAA) that has many applications, including back-trajectories and determination of the potential origin of air masses (Stein et al., 2015). Rolph et al., (2017) detail the READY (Real-time Environmental Applications and Display sYstem) web based version of the HYSPLIT model which is open source and available for public use.

Five days was chosen as the length of back trajectory modelling based on a conservative residence time of NH_4^+ in the atmosphere (Wasiuta et al., 2015b). A central location in the study area was chosen for modelling as the receptor site, though exact precipitation collection sites may differ slightly; for the general application of determining broad source regions of inorganic N the difference between modelled site and specific site should be well within the error of the model as the smallest grid size of available data is 12km x 12km. Central air mass sources were calculated at 500, 1000, and 2000m above ground level in an attempt to see potential distant and local sources of

inorganic N. Source areas for precipitation events on 9/1/2014, 10/4/2014, and 10/1/2016 were modelled.

Determination of Recent Glacial Retreat

Historical aerial photography was obtained for the Forget-Me-Not Glacier and its catchment through EarthExplorer (<https://earthexplorer.usgs.gov/>). The earliest images available were from 1952 so comparisons of ice extent were possible from 1952-2015. It should be noted that it is likely that the glacier was in retreat prior to 1952, if this glacier followed regional trends (Benedict, 1973; Graf, 1971; Meier, 1951), and that the 1952 extent should be viewed as a minimum for glacier extent area prior to this imagery. Using ArcGIS 10.3, changes in areal extent of ice were mapped to determine recently exposed pro-glacial areas. It should be noted that the lowest resolution imagery used is accurate to within ~23.6m and that viewing the imagery at scales greater than 1:47,200 may result in small spatial errors. Figures are displayed to negate this issue unless otherwise noted.

RESULTS

Snow Profile

Snow collected near the Jasper and Albino Lake study catchments (Figure 2.7 in Methods) on April 9, 2016 showed a nearly homogenous snowpack with depth in terms of temperature and crystal structure with rounds or rounding grains dominating the majority of the snowpack (Figure 3.1). A small sun-crust had developed on top of recently deposited snow; these two layers constituted the top 9 cm of the snowpack. The relatively homogenous nature of the snowpack indicates that the snow was likely representative of the precipitation that fell during the snow year.

Snow water equivalence measured at the Beartooth Lake SNOTEL Site (station 326), which is 4.5 km southwest from where the snow profile was measured, was at its highest snow water equivalent on April 24, 2016, at 18.7 inches (475mm) of water. Snow water equivalence at this SNOTEL site on the day the snow samples were collected was 17.2 inches (437mm) of water, thus representing 92% of the greatest snowpack water equivalence for the year. The large proportion of the peak snowpack indicates that the snow collected is representative of the snowpack as a whole
(<https://www.wcc.nrcs.usda.gov/snow/snotel-wedata.html>)

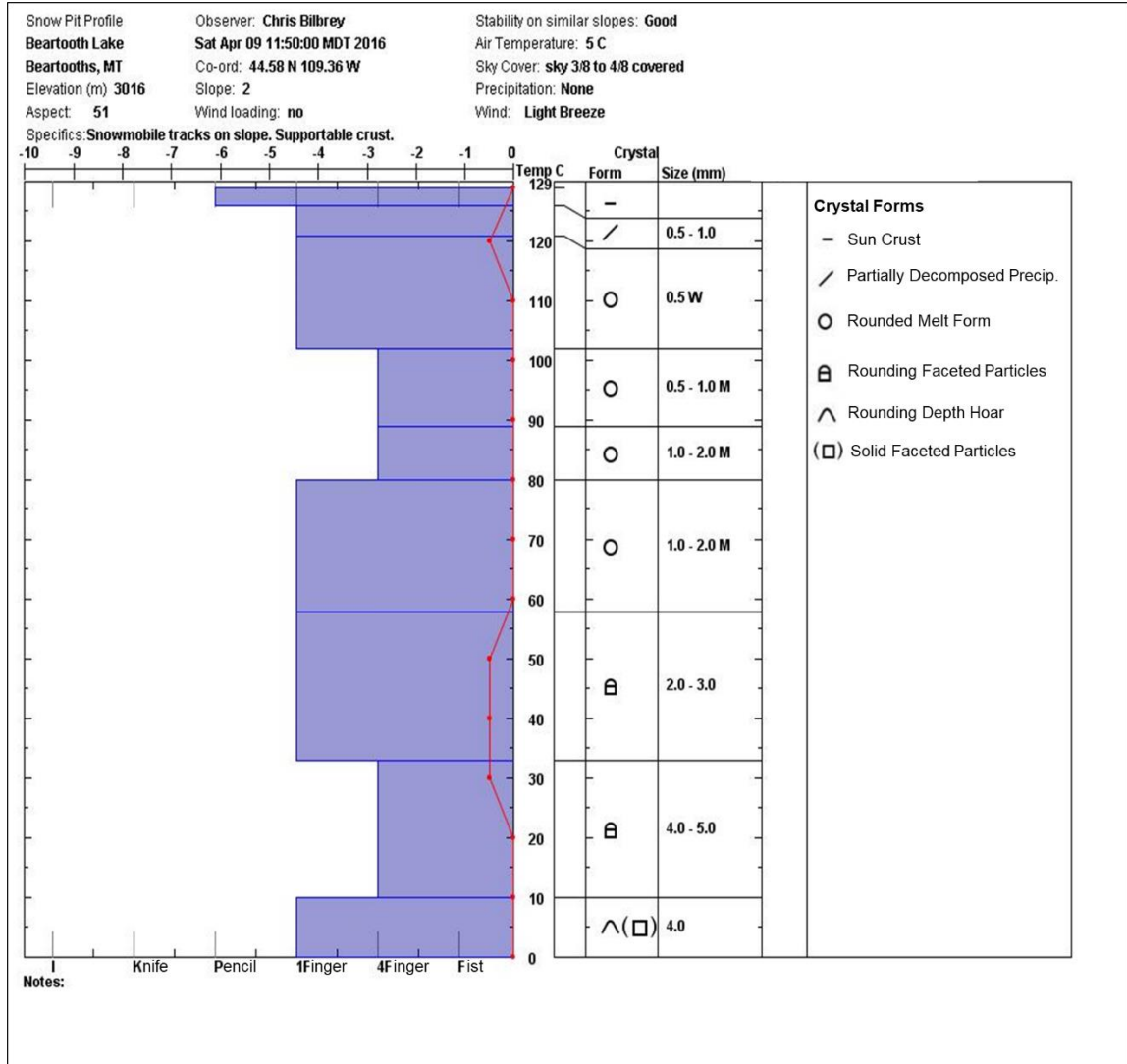


Figure 3.1. Profile of the snowpack collected from a snow pit at the location NNE of Clay Butte, north of Beartooth Lake (see Figure 2.7 in “Methods”) on April 9, 2016. The snow was nearly isothermal at 0°C throughout the ~ 129cm snow profile. The air temperature at time of sampling was ~7°C.

Water Measurements

Measurements of electrical conductivity (EC), and stream water temperature were taken at each of the water sampling sites on each of the sampling dates (Appendix Water

Measurements, Figure 3.2). pH was also measured but the probe gave inaccurate readings on days 214-215, 228-229, 238-239, 252-253, and 274-275 and therefore this data is not included. The highest upstream sites (sites 3340m, 3455m, and 3540m) were not visited on the earliest sampling dates (DoY 189 and 190) in the summer because of snow cover. Time series of the EC and temperature measurements are shown in Figure 3.2. EC values range from 1.5 to 12.9 μ S/cm. There is an increase in EC during the melt-season for the non-glacial site (NG) and the lower glacially fed sites (3150m, 3200m and 3340m), which is marked after DoY240. However, the higher sites (3455m and 3540m) and the Albino Lake stream only demonstrate a modest increase in EC during the earlier part of the melt-season, plateauing (or even slightly decreasing) near the end of the melt-season. The highest glacially sourced stream site consistently has the lowest conductivity, <5 μ S/cm (with one minor exception at day 252) (Figure 3.2). Water temperatures ranged from 1.0 to 11.3°C in the Jasper Lake catchments streams, with the Albino Lake Catchment stream ranging from 9.0 to 14.6°C. The water temperature measurements were taken at varying times of the day during the melt-season so direct comparisons are not possible; however, one clear trend is observable, that site 3055m, the lowest elevation, which is in the site Albino Catchment has the warmest waters during each sampling period. Sampling times are documented in Appendix Water Measurements.

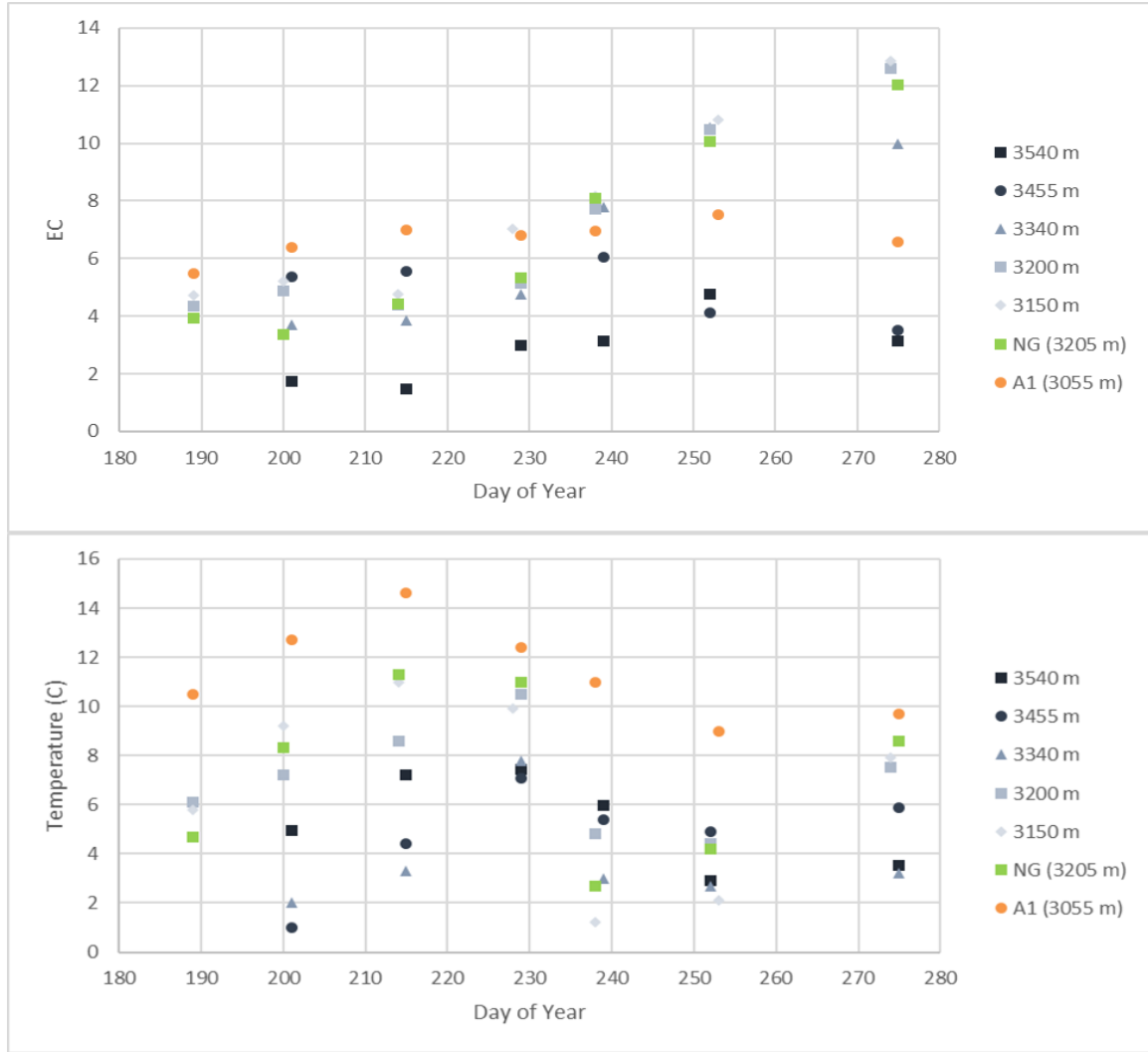


Figure 3.2. a) Time series of EC measurements of the Jasper and Albino Lake catchment streams. b) Time series of water temperature measurements of the Jasper and Albino Lake catchment streams. Error associated with the precision of the measurements is within the size of the symbol ($0.1\mu\text{S}/\text{cm}$ and 0.1°C respectively).

Stream Discharge

The discharge of the glacial outlet stream was measured once at each sampling time point during the melt-season (Table 3.1) at a location on stream 1, just downstream of the last major tributary before Jasper Lake (stream 2) ($\sim 100\text{m}$ below sites 3200m and 3205m – see Figure 2.2). Diurnal cycles in discharge are a common feature of glacially

fed streams (e.g. Benn and Evans, 2010) and such variations were observed in the glacially fed stream in the Jasper Lake catchment, although small lakes upstream from the measurement reach may modulate and reduce this effect. Discharge was measured in the morning on DoY 238 and 253, while all other discharge measurements were taken in the afternoon (Table 3.1). Since the time of day of discharge measurements varies by up to 6.5 hours, it is difficult to make robust seasonal comparisons.

Table 3.1. Discharge measurements throughout the melt-season, calculated using salt dilution gauging. Error for salt dilution gauging is typically within 5%.

Day Number	Time of Day	Q (m ³ /s)
229	14:00	0.213
238	11:00	0.071
253	11:15	0.003
274	15:40	0.005

Nitrate and Ammonium Composition of Stream Water and Snowpack

Stream Water Nitrate

Nitrate concentrations in the Jasper (glaciated) and Albino (unglaciated) catchments range from below detection limit (BDL), which 0.2µM, up to 120µM and BDL to 1µM respectively (Figure 3.3). Concentrations of nitrate at the low elevation stream sites (3150m, 3200m, and 3205m (ng)) in the glaciated catchment are up to 120µM higher when compared to the Albino Catchment stream. (Figure 3.3) These differences in nitrate concentration are amplified as the melt-season progresses; glacially sourced waters increase from ~20-40µM to 60-120µM, while the Albino (non-glacially sourced) waters remain low (<2µM) throughout the melt-season. The exception to this being waters at the highest site (3540m) of the glaciated catchment, where nitrate

concentrations do not show any significant increase as the melt-season progresses.

Interestingly, the nitrate concentrations in the stream in the Jasper Lake catchment that is not currently fed by glacial melt (ng) (site 3205m) mirror those in the glacially sourced stream at a similar elevation (Figure 3.3).

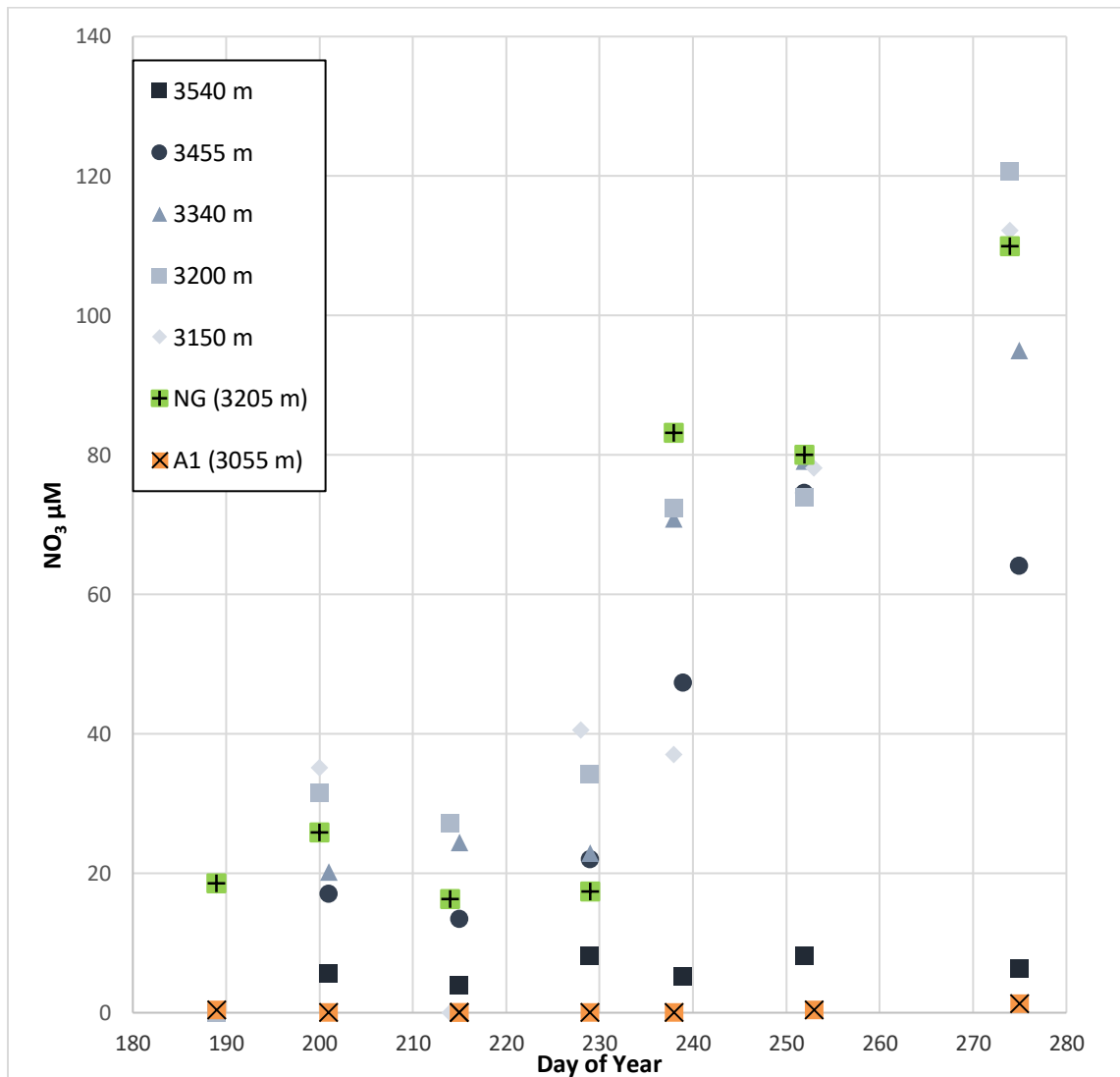


Figure 3.3. Time series of nitrate concentration measurements of the Jasper and Albino Lake catchment streams throughout the melt-season. Glacially fed waters have a blue color scheme while non-glacially sourced waters are shown in green and orange. Error associated with the precision of the nitrate concentration measurements is within the size of the symbol.

Stream Water Ammonium

Concentrations of ammonium are low, in the Albino Lake Catchment stream, present in only trace quantities, $<1\mu\text{M}$ (Figure 3.4). This is in contrast to the highest elevation glacially-sourced waters, i.e. closest to the glacier, where the highest site (3540m) has significant ammonium, up to $35\mu\text{M}$, but, with a notable decrease in ammonium concentrations below the highest site where concentrations never exceed $11\mu\text{M}$. Concentrations of ammonium show an increase as the melt-season progresses but only in the three highest glacially-fed sites, with this increase being greatest at the highest site (3540m). The seasonal increases in ammonium concentration are however, lower than those measured for nitrate by up to a factor of three (Figure 3.3).

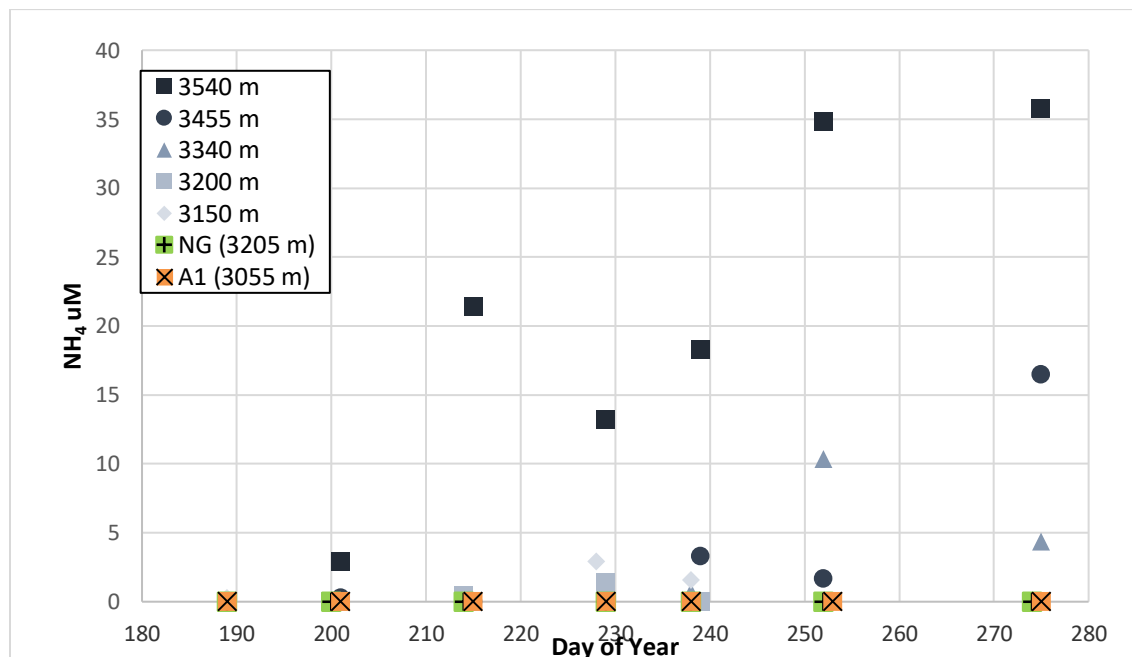


Figure 3.4. Time series of ammonium concentration measurements of the Jasper and Albino Lake catchment streams throughout the melt-season. Glacially fed waters have a blue color scheme while non-glacially sourced waters are shown in green and orange. Error associated with the precision of the ammonium concentration measurements is within the size of the symbol.

Snowfall and Snowpack

Ammonium concentrations ranged from 0.2-14.6 μ M in fresh snow and from 2.4-8.3 μ M in the snowpack (Table 3.2). Nitrate concentrations showed a similar range to the ammonium concentrations in fresh snow, 1.6-11.6 μ M, but higher values than ammonium for the snow pack, 4.6-27 μ M.

Table 3.2. Ammonium and nitrate concentrations in snow samples. See Figure 2.7 in Methods for snow sample locations.

Location	Date	NH ₄ (μ M)	NO ₃ (μ M)
Albino Lake	9/1/2014	14.6	11.6
Albino Lake	10/4/2014	2.1	1.6
Beartooth Lake (bottom of snowpack)	5/19/2015	8.0	13.4
Beartooth Lake (whole snowpack)	5/19/2015	2.5	17.2
Clay Butte	4/9/2016	5.6	4.6
Jasper Lake	7/18/2016	8.3	26.9
Jasper Lake	10/1/2016	0.2	2.4

Stable Isotopes of Nitrogen and Oxygen in Nitrate

The $\delta^{17}\text{O}$, $\delta^{18}\text{O}$, $\Delta^{17}\text{O}$, and $\delta^{15}\text{N}$ values of nitrate in stream water and atmospheric deposition (as snow) are shown in Figures 3.5, 3.7, 3.8 and 3.9- 9. Values are expressed using the δ and Δ notation (see Equation 1 and 2).

$$\delta = ((R_{\text{Sample}}/R_{\text{Standard}}) - 1) \times 1000 \quad (\text{Equation 1})$$

$$\Delta^{17}\text{O} \sim \delta^{17}\text{O} - 0.52 \delta^{18}\text{O} \quad (\text{Equation 2})$$

$\Delta^{17}\text{O-NO}_3$

$\Delta^{17}\text{O-NO}_3$ values show two distinct groupings (Figure 3.5). Atmospheric input from snowfall and as measured in the snowpack have highly elevated values when compared to stream water (glacially sourced or otherwise). The lowest $\Delta^{17}\text{O-NO}_3$ value

for snow is 12‰, while the highest value for stream water is 5‰, with stream water averaging between 1 and 2‰. The highest values for the stream water coincide with the start of the melt-season, when snowmelt contributions to the streams are at their highest, and show a distinct seasonal trend, declining as the melt-season progresses and the snowpack contribution to the streams decline.

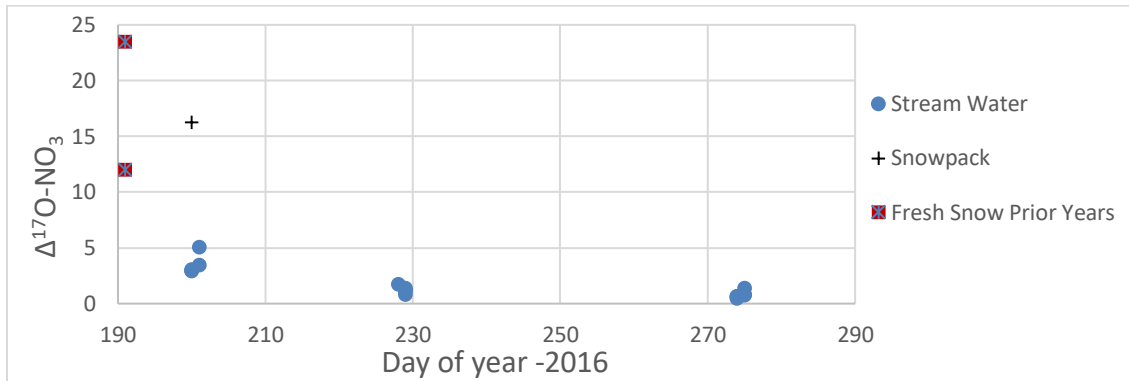


Figure 3.5. Time series of $\Delta^{17}\text{O-NO}_3$ measurements of the Jasper and Albino Catchment streams and atmospheric inputs (as snow). The precision of the $\Delta^{17}\text{O}$ measurements is $\pm 0.4\text{‰}$.

Following Michalski et al., (2004) the percent of total nitrate in stream water from atmospheric input was calculated using a two-component isotopic mixing model (Figure 3.6). The average $\Delta^{17}\text{O-NO}_3$ value of the atmospheric input into the system was 17.2‰, which is within the range of previously recorded $\Delta^{17}\text{O-NO}_3$ values from the atmosphere (Michalski et al., 2004, Savarino et al., 2007, Tsunogai et al., 2016), though the lowest of the three atmospheric $\Delta^{17}\text{O-NO}_3$ values from the field area was slightly lower than any found in the literature (see Michalski et al., 2003; Tsunogai et al., 2016). A $\Delta^{17}\text{O-NO}_3$ value of 0 was used as the other end member of the mixing model, following Michalski et al., (2003), who state that atmospheric nitrate with oxygen derived from either air O_2 or water have a $\Delta^{17}\text{O} = 0$. The percent of nitrate derived from the atmosphere in stream water

decreases as the melt-season progresses as the snowpack contribution to stream water also declines. Early in the melt-season DoY 200 the highest % of atmospheric nitrate is evident in the highest stream site (3450m), which is consistent with this site having the greatest snowmelt contribution at that time.

While only a sample of one, the non-glaciated catchment shows higher atmospheric influence on nitrate than the Jasper Catchment, which is consistent with much lower levels of nitrate being found in that catchment (see day 274 and 275 of Figure 3.6).

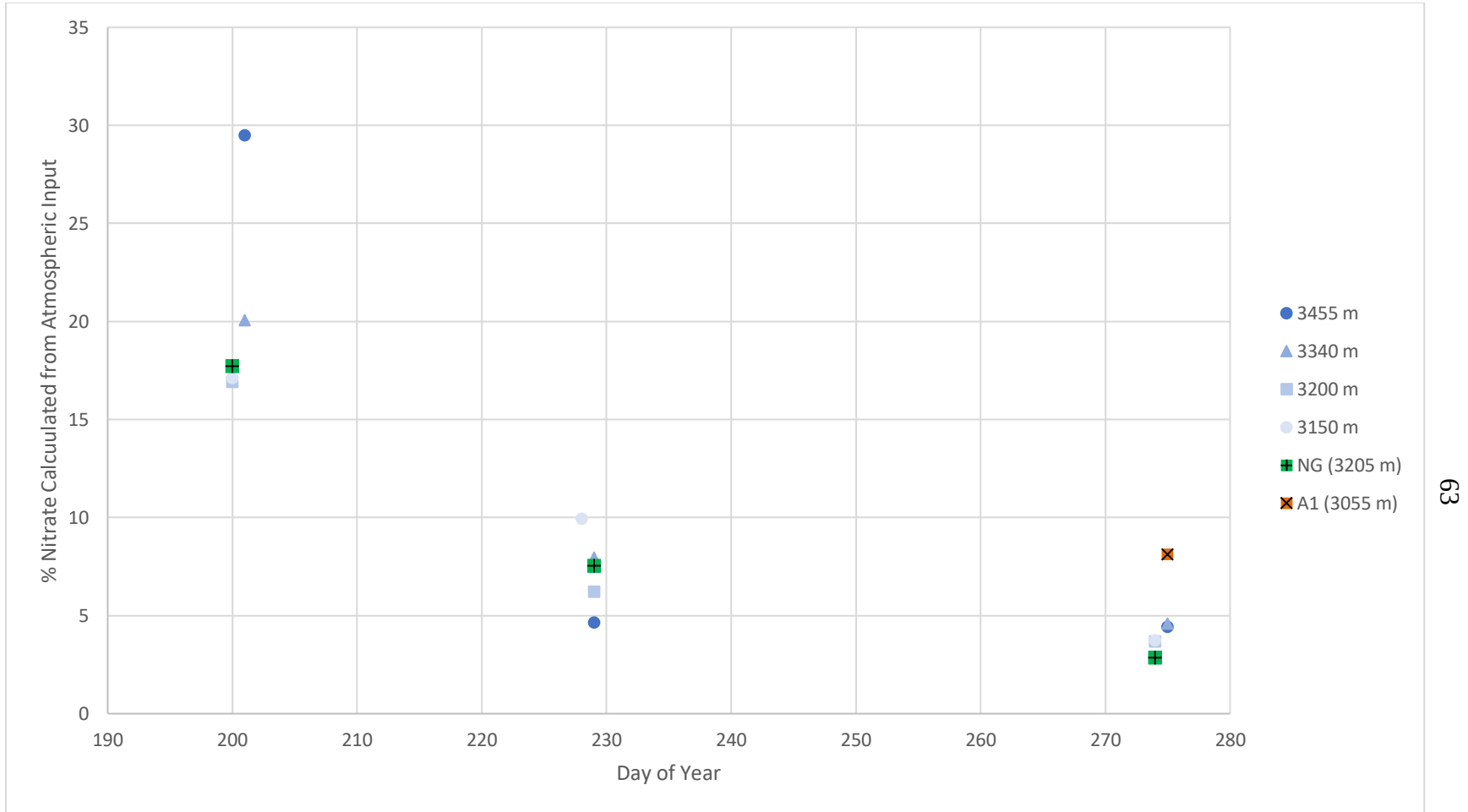


Figure 3.6. Time series of calculated atmospheric nitrate input of the Jasper and Albino Lake catchments streams throughout the melt-season. Glacially fed waters have a blue color scheme while non-glacially sourced waters are shown in green and orange. Error of nitrate derived from the atmosphere is based on $\Delta^{17}\text{O}$ measurements of both average atmospheric input ($17.2\text{‰} \pm 0.1\text{‰}$ (error based on precision of $\Delta^{17}\text{O}$ measurements and the number (3) of atmospheric observations)) and each stream water measurement ($\pm 0.4\text{‰}$, the precision of individual $\Delta^{17}\text{O}$ measurements). Percent atmospheric input error is $\pm 0.3\%$ (within the size of the symbol).

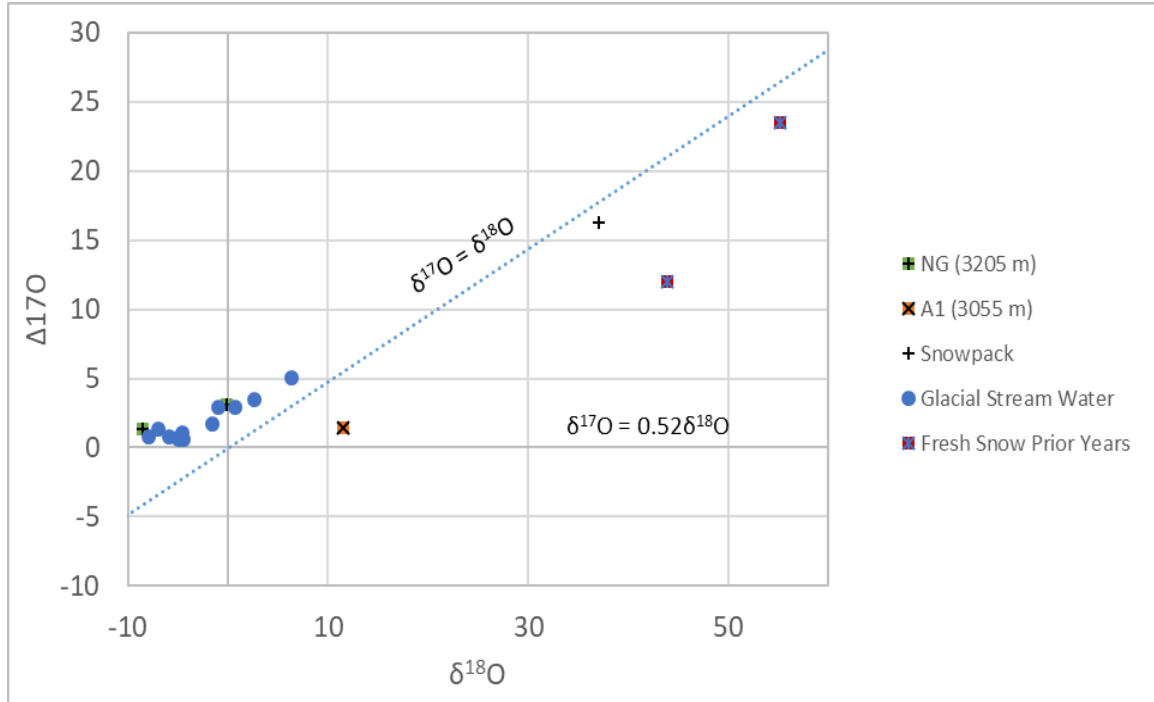


Figure 3.7. $\Delta^{17}\text{O}\text{-NO}_3$ vs $\delta^{18}\text{O}\text{-NO}_3$ values from the Albino and Jasper Catchments. Error associated with the precision of the $\Delta^{17}\text{O}\text{-NO}_3$ and $\delta^{18}\text{O}\text{-NO}_3$ values is within the size of the symbols.

$\delta^{15}\text{N}\text{-NO}_3$

$\delta^{15}\text{N}\text{-NO}_3$ values of snow range from -5.3 to -7.5‰ with $\delta^{18}\text{O}\text{-NO}_3$ values of 43.9 to 55.2‰ and thus they fall within the range of atmospheric NO_3 values on a co-isotopic plot (see Figure 3.8). Stream samples have $\delta^{15}\text{N}\text{-NO}_3$ values that are markedly higher (1.4 to 9.5‰) than the snowfall and snowpack samples, but this range of values is relatively constant during the melt-season (Figure 3.9).

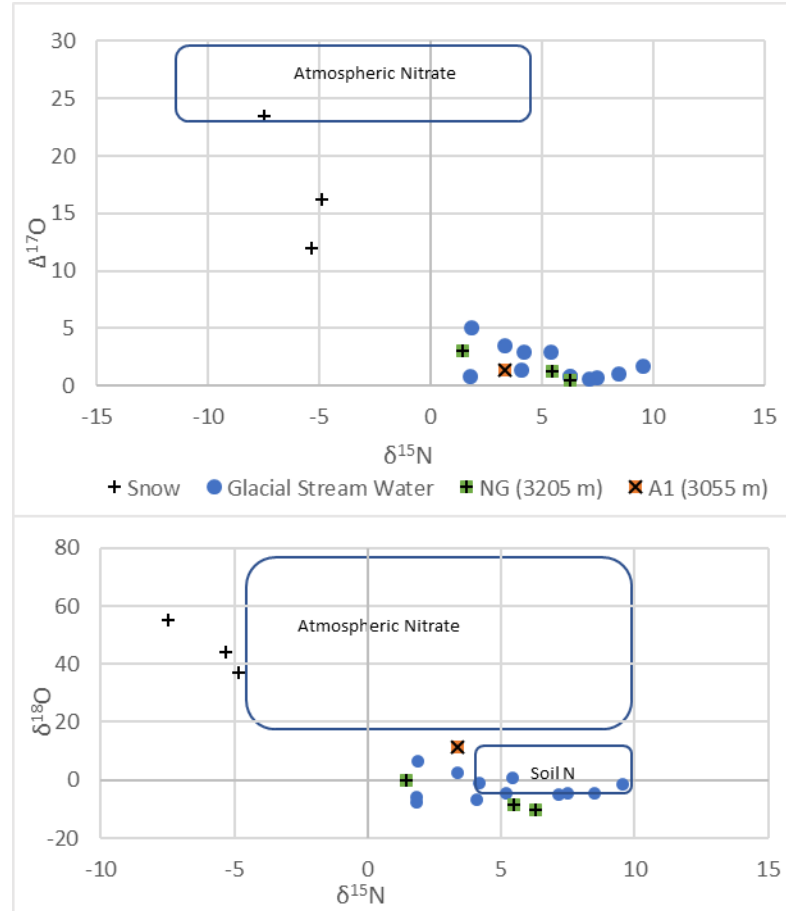


Figure 3.8. a) Identification of sources of nitrate using $\Delta^{17}\text{O}$ and $\delta^{15}\text{N}$ (box plots from Michalski et al., 2003). Glacially fed waters are shown in blue, non-glacially sourced waters are shown in green and orange. b) Identification of sources of nitrate using $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ nitrate values (box plots from Michalski et al., 2003). Glacially fed waters are shown in blue, non-glacially sourced waters are shown in green and orange. Error associated with the precision of the delta values is within the size of the symbols.

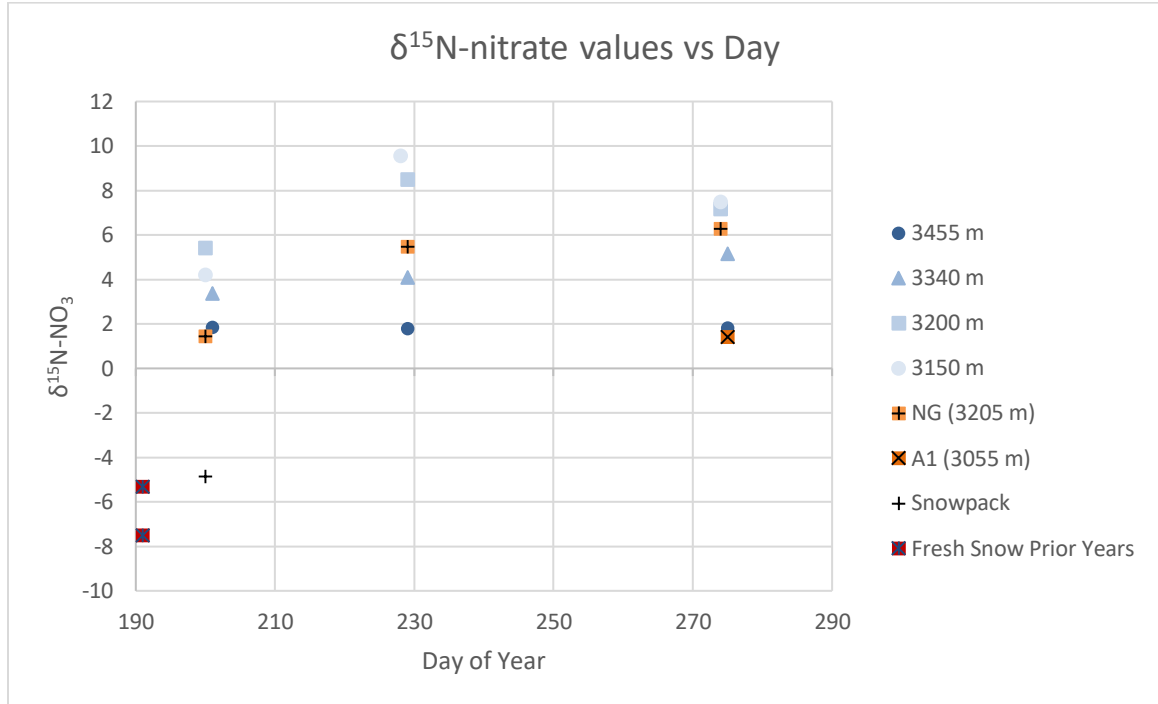


Figure 3.9. Time series of $\delta^{15}\text{N}$ -nitrate values in the Jasper and Albino Lake Catchment streams. Errors associated with the precision of the $\delta^{15}\text{N}$ values are within the size of the symbols.

Thin Section and XRF Analysis of Catchment Bedrock

A representative rock from the Jasper Lake Catchment was collected during the 2015 field season approximately 1km to the west of the Jasper Lake (Figure 2.2), and is a felsic granular gneiss, likely trondhjemite based on high Na to Ca ratios, see Table 3.3. Thin section analysis of this gneiss indicates that it contains a large amount of potassium rich minerals, mainly microcline, orthoclase, and biotite. These potassium rich minerals are potential sites for some replacement of ammonium for potassium (Bebout et al., 2016). Unnormalized weight percent results show that the granular gneiss contained nearly 3% potassium by weight (Table 3.3). Myrmekite (quartz intergrowths with plagioclase) and sericite were also present and may indicate metasomatic conditions that

could be consistent with the introduction of NH_4^+ into cation positions usually held by K^+ (Kehelpannala and Ratnayake, 1999, Maineri et al., 2003). Previous work (Bebout and Fogel, 1992) has shown a positive correlation between K_2O and N concentrations in other metamorphic rocks (grade does not appear to affect the relationship), such that a rock containing 2.78% K_2O should also contain between 100 and 600ppm N. If the N, is organically sourced, then significant amounts may devolatilize at temperatures from ~350-550°C, resulting in a change in the $\delta^{15}\text{N}$ values in the rock by between +2 to +5‰. (Bebout et al., 2016).

Table 3.3. Major elements in unnormalized weight percent of the felsic granular gneiss sample, representative of the catchment bedrock. LOI is Loss On Ignition.

	Unnormalized Major Elements (Weight %):
SiO₂	74.01
TiO₂	0.16
Al₂O₃	14.55
FeO*	1.23
MnO	0.01
MgO	0.22
CaO	1.71
Na₂O	4.45
K₂O	2.78
P₂O₅	0.02
Sum	99.13
SO₃ >/=	0.01
Cl >/=	0.00
LOI %	0.55

Three rocks sampled from the proglacial area below Forget-Me-Not Glacier during the 2016 field season were also analyzed via thin section. The first two were sampled because of their broad representation of the majority of the rocks in the Jasper Catchment and are also felsic granular gneisses similar to the sample collected in 2015. The third sample which represented only a small proportion of the rocks in the proglacial

area was an amphibolite, likely a product of metamorphosed mafic dikes that intruded the area between 1.3 Ga and 780 Ma (Henry and Mogk, 2003). The presence of sericite in all three of the proglacial samples is a further indication of past metasomatic conditions (Maineri et al., 2003). A full description of thin sections is given in the Appendix Thin Sections.

Abiotic Weathering Laboratory Experiments

Abiotic weathering of five distinct samples of finely crushed unoxidized bedrock when reacted with deionized water (to simulate glacial ice melt) at 4°C showed a wide range of initial ammonium release. The release of ammonium after five minutes reaction time of the milled rock with deionized water ranged from 9 to 69µM (Figure 3.10). Ammonium concentrations in the five samples tended to converge as time progressed in the 330 hour experiment. Samples with lower initial concentrations tended to increase over the 330 hours, whereas samples with higher initial concentrations tended to show a decrease over the same time frame. Concentrations of ammonium after 330 hours ranged from 26 to 46µM (Figure 3.10, Figure 3.11) with dissolved inorganic N (DIN - the sum of ammonium, nitrite and nitrate) concentrations only slightly higher ranging from 28 to 53µM (Figure 3.11).

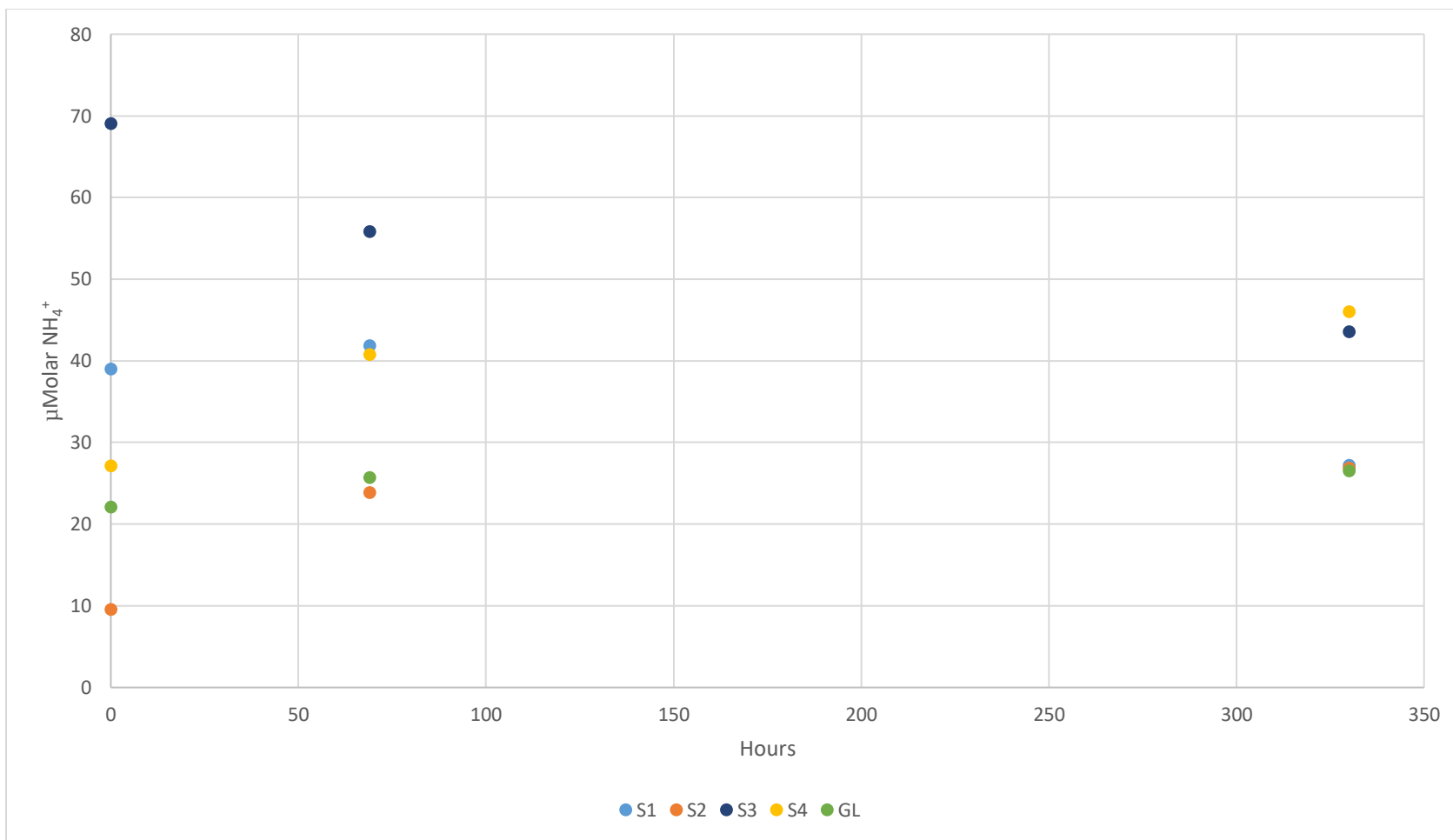


Figure 3.10. Ammonium production from finely milled unoxidized catchment rocks during 330 hours of exposure to deionized water on a shaker at 4°C under aerobic conditions. S1-4 were rock samples collected from the proglacial area, where S1 and S4 are amphibolite, and S2, S3, and GL are felsic granular gneiss. Standards were run in parallel with the samples for major cations at concentrations of 0.01, 0.05, 0.1, 0.5, 1, 10, and 20ppm. Cation concentrations varied less than 0.1ppm and linear regression of known cation concentrations and outputs showed R^2 values of 0.998 or greater.

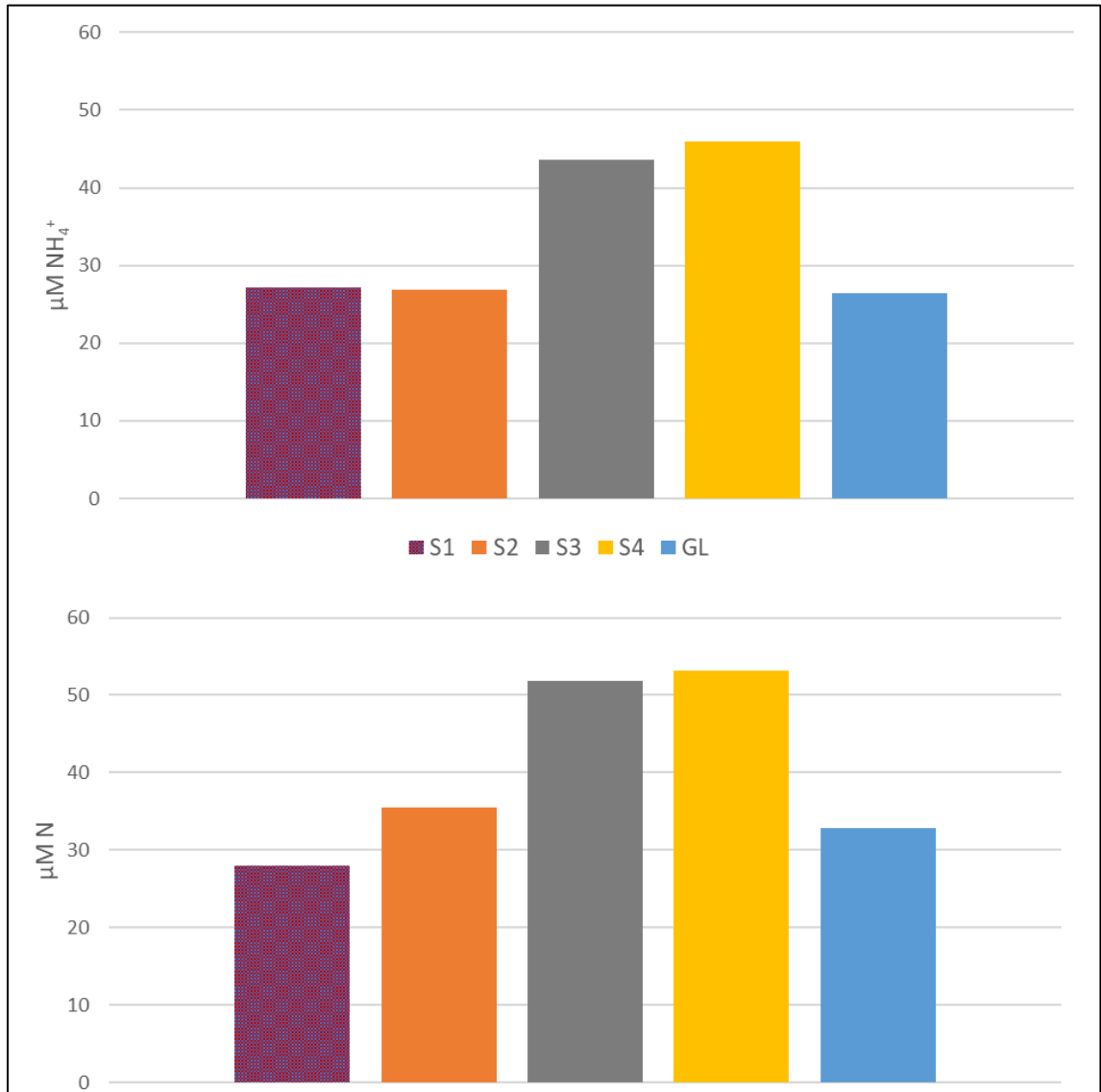


Figure 3.11. Inorganic N production from finely milled unoxidized catchment rocks after 330 hours of exposure to deionized water on a shaker at 4°C under aerobic conditions. a) Ammonium concentrations b) Dissolved inorganic N concentrations (sum of ammonium, nitrite and nitrate). Standards were run in parallel with the samples for major cation and anions at concentrations of 0.01, 0.05, 0.1, 0.5, 1, 10, and 20ppm. Cation concentrations varied less than 0.1ppm and linear regression of known cation concentrations and outputs showed R^2 values of 0.998 or greater. Anion concentrations varied less than 0.1ppm and linear regression of known cation concentrations and outputs showed R^2 values of 0.999 or greater.

The small difference between the DIN and ammonium concentrations indicates that a much greater proportion of the water soluble inorganic N in the bedrock (averaging 81%) is stored as ammonium, though all samples contained some bedrock nitrite and nitrate.

Enrichment Culturing Results

Nitrite Enrichment Results

Nitrate concentrations in the nitrite enrichment cultures incubated at 4°C showed no detectable increase, staying below 6 μM throughout the 97 day incubation (Figure 3.12). A minor increase in nitrate concentration (1.5 μM) occurred in the uninoculated media over the 97 day incubation, but was not significant enough to be considered as evidence of contamination.

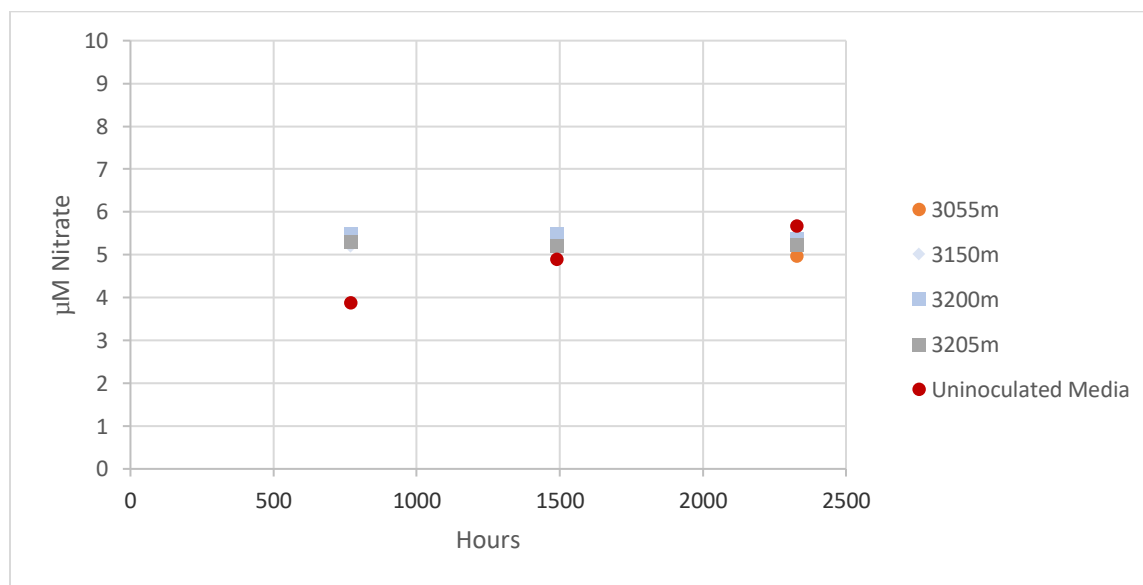


Figure 3.12. Nitrate concentrations in the nitrite oxidizer enrichment cultures, during a 97 day incubation at 4°C. Error associated with the precision of the nitrate concentration measurements is within the size of the symbol?

Ammonium Enrichment Results

There was no significant difference in the ammonium concentrations in the samples containing native microbes and amended with the ammonium enrichment media, and the uninoculated media when incubated at 4°C for 97 days. Both the inoculated samples and uninoculated media showed a decline of ~130 μ M ammonium over the 97 day incubation, with final concentrations of 1714 μ M (average) and 1710 μ M respectively (Figure 3.13) and is possibly a result of contamination. The outlying value at site 3055m after 1500 hours incubation is likely erroneous as the values for the site 3055m incubations are consistent with other samples containing native microbes both before and after this time point.

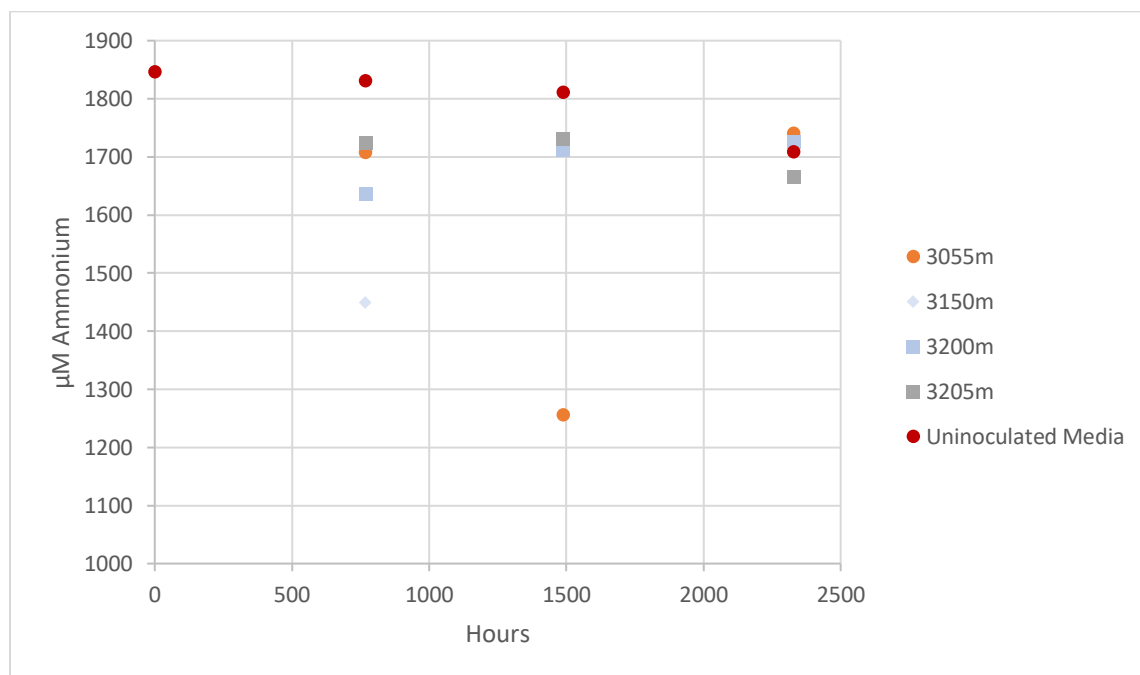


Figure 3.13. Ammonium concentrations in the ammonium oxidizer enrichment cultures, during a 97 day incubation at 4°C. Error associated with the precision of the ammonium concentration measurements is within the size of the symbol.

16S rRNA Gene Sequencing Results

The top ten phylotypes by relative abundance, based on partial sequencing of the 16S rRNA gene (base pairs 1100-1492) are shown at each individual site (Appendix Bacterial 16S rRNA gene library compositions). Where a cultured representative for the phylotype exists in the National Center for Biotechnology Information (NCBI) data base they are noted. Note that three phylotypes associated with humans (those most closely related to *Streptococcus pneumoniae*, *Cutibacterium acnes*, and *Staphylococcus epidermidis*), make up a significant proportion of the total 16S rRNA gene phylotypes in the non-glacial catchment site. This site is the closest to the trailhead and therefore likely experiences the greatest proportion of human traffic. Such phylotypes are not present in the top ten most abundant phylotypes samples from the more remote sites, i.e. at higher elevations, in the glaciated catchment.

The closest cultured representatives for the non-human associated phylotypes found in the non-glacial and glaciated catchments are generally not surprising for these environments. The cultured representatives (*Massilia psychrophilum*, *Flavobacterium sp.* strain RB1R5, *Polaromonas sp.* strain W13N, *Massilia sp.* 5S-42, *Alkanindiges illinoisensis* strain HYN0046, *Burkholderia sp.* strain KBL32) are typically associated with rock, soils, or biota found in glacial, polar, or alpine environments (Coenye and Vandamme, 2003; Appendix Bacterial 16S rRNA gene library compositions). Short descriptions of these bacteria and references therein are in Appendix Bacterial 16S rRNA gene library compositions.

The percent of total bacterial diversity that the top 10 phylotypes account for, in general, increases as distance to the glacier decreases (Appendix Bacterial 16S rRNA gene library compositions). This suggests that bacterial community structure changes along the glacial stream.

Presence of Nitrifiers

Phylotypes related to known nitrifiers (order Nitrosomonadales or Nitrospirales) were present in samples from both the glaciated and non-glaciated catchments (Figure 3.14). These phylotypes only accounted for a small proportion (0.01 – 0.35%) of the total 16S rRNA gene sequences for any given sample. Additional details on the closest matches for the putative nitrifier phylotypes from the NCBI database are presented in Appendix A, Bacterial 16S rRNA gene library compositions (Table A1).

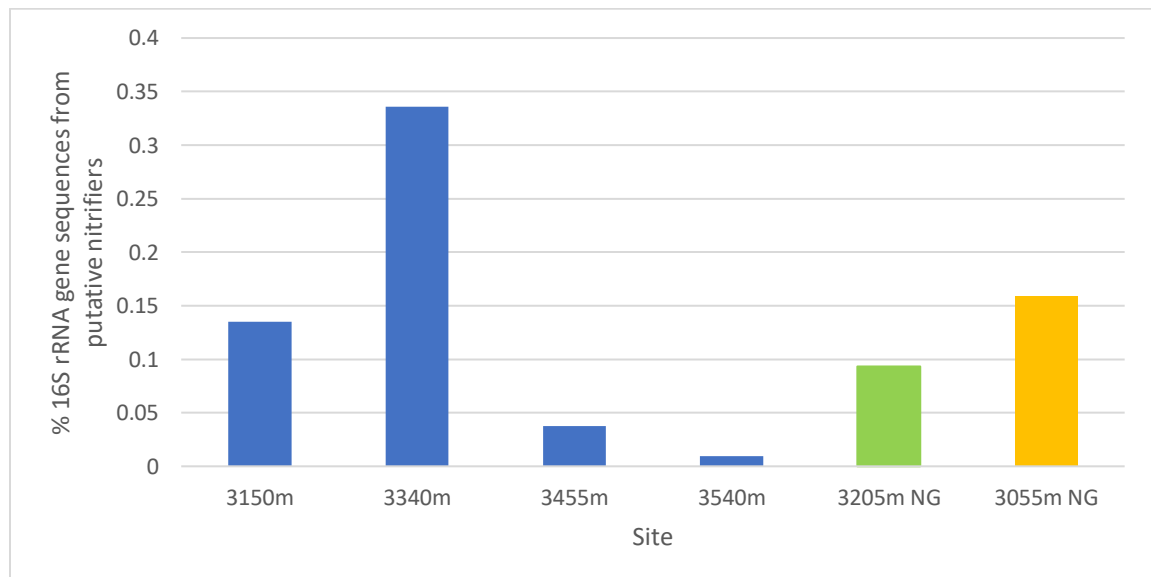


Figure 3.14. Relative percent contribution of putative nitrifiers, to total sequences, based on partial sequencing of the 16S rRNA gene (1100-1492 base pairs). “NG” refers to sites that are not currently sourced by glacial ice.

Table 3.4 Nitrifier presence and copy densities from stream samples on DoY 275. “NG” refers to sites that are not currently sourced by glacial ice.

	3150m	3340m	3455m	3540m	3205m (ng)	3055m (ng)
Relative Percent of Putative Nitrifiers compared with total sequences	0.14	0.34	0.04	0.01	0.05	0.16
Copy Number of Putative Nitrifiers/ Liter Stream Water	5.5E+4	9.5E+4	8.9E+4	2.3E+4	1.9E+4	6.4E+3
Total Copies/ Liter	4.1E+07	2.8E+07	2.4E+08	2.5E+08	3.5E+07	4.1E+06

Ion Fluxes (Nitrate and Ammonium) for the Jasper Lake Catchment

The instantaneous flux of specific N species (nitrate and ammonium) was calculated using the concentration of that ion measured at site 3150m (Table 3.4 and Table 3.5) and the calculated stream discharge determined via salt slug method (Table 3.1). The seasonal flux of the N species was estimated by averaging the flux between temporally adjacent measurement times and summing the flux based on those intervening time periods (Equation 3, Tables 3.6 3.7, and 3.8).

$$Seasonal\ flux = \Sigma \left(\left(\frac{flux1 + flux2}{2} \right) * (time2 - time1) \right) + \left(\left(\frac{flux2 + flux3}{2} \right) * (time3 - time2) \right) + \dots \quad \text{Equation 3}$$

This flux estimate assumes that instantaneous flux calculations are representative of the period of the melt-season they were measured, and not influenced by time of day or recent precipitation. Instantaneous flux calculations assume that measured N concentrations at site 3150m is the same as at the stream discharge site ~250m upstream.

Table 3.5. Instantaneous nitrate flux, passing through the stream discharge site for specific times and dates during the melt-season.

Day #	Time	μM nitrate 3150m	Q (m^3/s)	nitrate flux ($\mu\text{mol/s}$)
229	14:00	40.5	0.213	8646
238	11:00	37.1	0.071	2629
253	11:15	78.2	0.003	230
274	15:40	112.2	0.005	602

Table 3.6. Instantaneous ammonium flux, passing through the stream discharge site for specific times and dates during the melt-season.

Day #	Time	μM ammonium 3150m	Q (m^3/s)	ammonium flux ($\mu\text{mol/s}$)
229	14:00	2.9	0.213	620
238	11:00	1.6	0.071	109
253	11:15	0	0.003	BDL
274	15:40	0	0.005	BDL

Table 3.7. N species flux/season showing the estimated seasonal flux of N species using the salt slug discharge method.

Moles nitrate flux/season (day 229-274)	Moles ammonium flux/season (day 229-274)
6993	710

Table 3.8. N flux/season showing total N flux for the glacial stream at the discharge site

Moles N flux/season (DoY 229-274)
7703

Table 3.9. N flux/day showing moles of N transported/day averaged over the time of respective measurements.

Moles N/day (DoY 229-274)
157.2

Jasper Lake Catchment N Input (Snowpack and Precipitation)

The contributing area above the discharge site in the Jasper Lake catchment is $\sim 2829000\text{m}^2$ (calculated using GIS) and the total snow fall for the 2015-2016 snow year from the Beartooth Lake SNOTEL Site (see Figure 2.2 and 2.7) was 536mm of snow water equivalence. Assuming snowfall for the Jasper Lake Catchment was the same as for the SNOTEL site (8 km to the SW) then there was $\sim 1516000\text{m}^3$ or $1.516 \times 10^9\text{L}$ of snow water equivalence added to the Jasper catchment above the discharge site during the 2015-2016 snow year. Using an average concentration of $8.5\text{ }\mu\text{M L}^{-1}\text{ N}$ for snow (from samples collected in both the snow year and melt-season) (Table 3.2) an estimate of the total N deposition for the 2015-2016 snow year can be calculated, 12889 moles of N. If total precipitation is used (not just snowfall) to calculate the N depositional flux, there was 681mm of water equivalence at the Beartooth Lake SNOTEL Site, for the entire year, and thus for the catchment this would result in a 1926000m^3 ($1.926 \times 10^9\text{L}$) for total precipitation for the water year. Assuming that the liquid precipitation has the same N concentration as the snowpack, $8.5\mu\text{M L}^{-1}$ then the total annual wet deposition of N for the catchment in 2015-2016 was $\sim 16,400$ moles.

A comparison of the annual molar N flux values from the catchment, $\sim 7,700$ (Table 3.7) relative to the catchment atmospheric input 16,400 moles N, shows that there is potentially a discrepancy, in order to account for the output flux values at the considering that the stream N flux estimate is only for a 45 day time period. This suggests that there is likely a source of N to the catchment in addition to atmospheric input, most likely from bedrock/sediment weathering.

With an average release of $40\mu\text{M}$ N for every 7g of sediment in 10ml of DIW (outlined in Methods, Abiotic Weathering) then one gram of unoxidized sediment produces $0.057\mu\text{M}$ of N. Assuming that the N production can be scaled up then each kg of ground sediment could release $57\mu\text{M}$ of N.

Atmospheric Modelling of Inorganic N Deposition

An analysis of air mass movement (i.e. its trajectory) in the days prior to specific precipitation events, September 1, 2014, October 4, 2014, and October 1, 2016 can provide information on the potential source regions for the inorganic N that was deposited in the snow in the study area. HYSPLIT (described in Methods, Modelling of Air Masses Supplying Precipitation to the catchments) results can be seen in Figure 3.15.

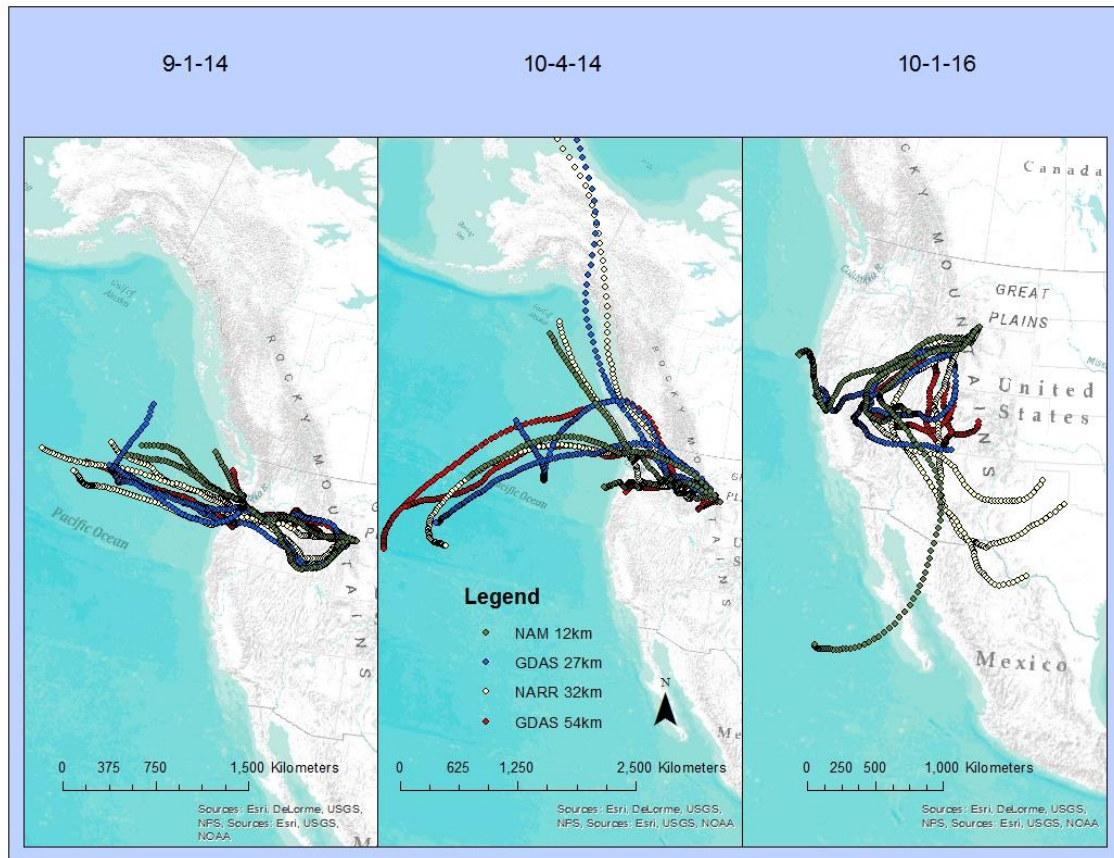


Figure 3.15. Modelled source tracks for the air masses over the five days prior to precipitation (snowfall) occurring in the study catchments in the Beartooth Mountains. The different colors represent different models with varying grid size see legend. Base imagery from ESRI.

Sources of N relevant to the precipitation events of September 1, 2014, October 1, 2014, and October 1, 2016 could include industrial/urban sources such as the Bay Area, Salt Lake City, Portland, or Seattle; agricultural/rural sources could include southern Idaho and eastern Washington.

Models of emission sources of N into the atmosphere in the continental United States have been published (e.g. Zhang et al., 2012). Urban areas that may contribute N (usually in the form of NO_x) to the study area (e.g. the Bay Area, Salt Lake City,

Portland, and Seattle) produce large (up to $50\text{kg N ha}^{-1} \text{ a}^{-1}$) while agricultural areas that may contribute N (usually in the form of NH_3) to the study area (e.g. southern Idaho and eastern Washington) produce $\sim 25\text{kg N ha}^{-1} \text{ a}^{-1}$.

DISCUSSION

It has been demonstrated that the streams draining certain presently glaciated catchments in the Beartooth Mountains of south-central Montana (Figure 1.2) contain nitrate concentrations up to an order of magnitude greater than the streams draining non-glaciated catchments, with similar areas, elevations, lithology, macro flora and fauna, atmospheric inputs, and comparable downstream lakes (Saros et al., 2010; Slemmons et al., 2015). To examine the impacts of glaciers on alpine nitrate production, this study uses a paired catchment system where two catchments are as similar as nature allows, except for one variable (in this case the presence of a glacier in one of the catchments, and the absence of perennial ice in the other); this difference between catchments allows for the effects of that variable to be examined. The difference in nitrate concentrations between the catchment types (glaciated or unglaciated), implies that there are different sources of nitrate, or unique mechanisms acting, between the catchments that is dependent on the presence of a glacier.

Previous research has examined the differences in nitrate concentrations in the streams draining SF and GSF catchments with measurements being taken at the stream mouth, where waters enter the lake (Saros et al., 2010; Slemmons et al., 2015; Williams et al., 2007). However, no analysis has been undertaken that assesses the downstream changes in nitrate and other N species (e.g. ammonium) from the glacier source to the lake input location. This study examined the downstream changes in nitrate and other N species in stream water at five locations from the terminus of the Forget-Me-Not Glacier to the input to Jasper Lake, the first large waterbody (area $\sim 278,000\text{m}^2$), (Figure 2.11).

Furthermore, glacial waters and controls (stream inputs into the snow fed Albino Lake (area $\sim 359,000\text{m}^2$), and the non-glacially sourced tributary in the Jasper Catchment) were sampled for the entirety of the melt-season. The melt-season for the summer of 2016 was defined as the timing of the first sampling, when the stream sampling sites could be reached after they were no longer snow covered until significant snowfall occurred in the fall. Note, the lowest sites, up to 3205m, were sampled starting on DoY 189, while the highest three glacially sourced sites were not accessible until DoY 200 and the last sampling for all sites took place on DoY 275. Sites were sampled approximately every two weeks (9-15 days) during this time frame. The N flux from the glaciated catchment was also estimated using measurements of stream discharge taken at a single point on the glacially sourced stream and using measurements of ammonium and nitrate concentrations of the stream water from 275m m downstream of that location.

Potential differences in the nitrate sources by catchment type may be geologic (i.e. enhanced weathering of N bearing rocks from glacial processes), atmospheric (i.e. storage of atmospherically deposited N that is released from glacial ice during glacial retreat), or biologic (i.e. enhanced nitrate production through nitrification in glacial or periglacial environments). This study aims to investigate the relative importance of these factors that result in the enhanced nitrate concentrations observed in the catchment that is currently glaciated.

Stream Water: EC, NO₃⁻ Concentration, NH₄⁺ Concentration, and N FluxEC

The electrical conductivity of the stream water in the glacier fed catchment increased as the discharge decreased over the course of the melt-season (Figure 3.2 and Table 3.1). This relationship has been commonly observed for glaciated catchments (e.g. Collins, 1977).

NO₃⁻ Concentrations

In general, nitrate concentrations were progressively higher in the waters sampled at locations lower in the catchment, i.e. as the distance from the glacier increased. This may be in part to the continued oxidation of ammonium in waters, though there must be additional N sources based on the amount of added nitrate to the system.

Significant increases in nitrate concentration were not observed over the period July-October (DoY 189-275) at the snowmelt fed Albino Lake catchment site (3055m) and the maximum nitrate concentration was 2µM (Figure 3.4). The highest glacial-fed stream sampling site (3540m) also exhibited only modest nitrate concentrations, with a maximum value of 9µM (Figure 3.3), with no discernable increase in nitrate concentration as the melt-season progressed.

The increase in nitrate concentrations in glacial stream waters may also be a result of stream discharge decreasing as the melt-season continued. A large increase in nitrate concentrations of the glacial waters downstream of the 3540m site was observed on DoY 239, which corresponds to a decrease in stream discharge of 66% (Table 3.1). The

instantaneous N flux generally decreased through the melt-season as discharge decreased, but precipitation just prior to the last discharge measurement on DoY 274 likely caused a notable increase in discharge at the end of the melt-season accompanied by a rise in total N flux (Table 3.4).

Previous research on nitrate concentrations in streams draining rock glaciers in the Colorado Rockies showed “trough” like trends with high concentrations early and late in the melt-season (Williams et al., 2007). It is possible that with a later start date for data collection in this study, initial high concentrations of nitrate in stream waters at low discharges at the very start of the melt-season were missed. Alternatively, the high nitrate concentration at the start of the melt-season measured in the Colorado Rockies which was attributed to an “ionic pulse” from concentrated snowpack, (Williams et al., 2007) may not have occurred in the study area.

NH₄⁺ Concentrations

Ammonium concentrations in stream waters exhibited a significantly different spatial pattern than nitrate concentrations. Concentrations of ammonium were low (3μM) in stream waters at the glacial terminus (site 3540m) on DoY 200, early in the melt-season, but then showed an increase from DoY 214-274, (range 13 – 35μM, Figure 3.4). The increase in ammonium concentrations at the glacier terminus site occurred when the source waters for the stream transitioned from snowmelt to ice melt based on field observations. The increase in ammonium concentrations during the melt-season is concurrent with a decrease in discharge over the same time period (Table 3.1) and likely reflects lower flows in the subglacial drainage system. The increased water residence

times, lead to increased water-rock contact times and thus the potential for enhanced (biogeochemical) mineral weathering supplying greater amounts of NH_4^+ to the stream water. There is significant glacially-derived finely comminuted sediments immediately upstream from site 3540m which provides a large reactive surface area. This high reactive surface area of relatively unoxidized rock flour, will increase the surface exposure of NH_4^+ in mineral crystal lattices (Anderson, 1997; Holloway et al., 2002). The relatively high and increasing ammonium concentrations at site 3540m during the melt-season coupled with consistently low nitrate concentrations (Figure 3.3), indicates that the oxidation of NH_4^+ to NO_3^- occurs downstream from site 3540m.

Stream waters at other sites showed minor increases in ammonium concentrations, with maximum values of $16\mu\text{M}$ and $10\mu\text{M}$ at the next two highest sites (3455m and 3340m) respectively, while concentrations at the other sites were always below $3\mu\text{M}$. As for nitrate, it is possible that higher early season concentrations of ammonium were missed due to the study sampling time period.

Concentrations of ammonium are too low to account for all of the nitrate that is observed in stream 1 (Figure 3.3 and 3.4). Therefore there must be a source for the nitrate in the stream water that is in addition to the input from geologic and atmospheric sources. Biologic N_2 fixation may be the additional process that provides sufficient fixed nitrogen that is ultimately converted, likely microbially, to account for the elevated nitrate levels observed in the glacial catchment. Bacteria capable of N_2 fixation (e.g. *Polaromonas spp.*) have been found at all sites (Table A2), and may be providing a source of fixed N.

The ammonium contribution to total N flux at the discharge site, was 10 to 74 times lower than the nitrate contribution (Table 3.6) and the increase in the N flux on DoY 274 was solely based on nitrate, as ammonium concentrations were below detection limit on that date (Table 3.4 and 3.5).

N Flux

Concentrations of nitrate increase through the melt-season, which corresponds to a decreased N flux per unit time and decreased discharge (Tables 3.4-3.7), in the lower elevation glacially fed waters. It would appear that once there is no longer a large supply of snow, and glacial ice takes becomes a larger contributor to water supply in the stream in the Jasper Catchment, the capacity of the stream water becomes the limiting factor on N flux through the catchment. N flux appeared dependent on discharge, with more water transporting more N, even when concentrations of N were low.

N Sources in the Field

Geologic

NH_4^+ replacement of K^+ in crystal lattices of micas and feldspars may provide a site for N in rocks found in the catchment (Boyd et al., 1993; Busigny et al., 2004).

Abiotic weathering experiments showed that unoxidized, freshly ground, rocks sampled from the proglacial area in the Jasper Lake Catchment (Figure 2.2) could produce on average 57 μmoles of readily water soluble N per kg of rock. However, this calculation is

based on the entire rock mass being reduced via mill to a grain size of $3\mu\text{m}$. The unsorted sediment in the catchment proglacial area will have a much lower specific surface area. If only the proglacial area since 1952 is considered as a source for geologic N, then sediment with a porosity of 30% (average for glacial till (Nilsson et al., 2001)) would have to be 1.3m deep in order to produce the N flux estimate calculated for the glacial catchment at the discharge site. The glacier is certainly not producing this much sediment annually as there are no morainal features near the terminus of the glacier despite very little retreat since 1980 (Figure 4.5). Abiotic rock weathering experiments did not test for release of N after water had initially run through the system, so release of N may persist for some period as water continues to flow through sediments, though release of N is bound to decrease after some time. There are inherent limitations to this calculation of sediment thickness such as the real specific surface area of reactive minerals in the proglacial area, the accurate delineation of the proglacial area as being representative of the region of mineral weathering and that the laboratory experiments do not accurately mirror natural weathering processes. Therefore, it represents likely an “order of magnitude” estimate of the amount of sediment needed to account for the estimated flux of N downstream.

Field observations show an uneven distribution of sediment in the proglacial area such that there were regions where the sediment was potentially many meters thick (Figure 4.1 and Figure 4.2) and also places where there was no sediment with glacial polish visible on the bedrock surface (Figure 4.3). Therefore, it seems unlikely that if the sediment thickness was averaged for the entire proglacial area that it would exceed 1.3m.



Figure 4.1 Glacial sediment in the proglacial area 400m from the glacier terminus. The large boulder in the center-right of the photo is ~1m tall.



Figure 4.2 Pools in the proglacial area (250m downstream from the glacier terminus) that are a likely sediment sink and that were always inundated with water during the sampling period.



Figure 4.3 Glacially molded bedrock indicated by striations which are parallel to the paleo ice-flow direction. The photograph was taken 150m due east of the modern glacier terminus and the paleo flow of glacial ice was parallel to the modern day stream but is nearly perpendicular to modern glacial flow direction.

There is also a large amount of sediment directly down valley from the immediate proglacial area that likely maximizes water-sediment interactions (Figure 4.4). Here the stream completely disappears into poorly sorted material directly down valley from glacial terminus position in 1952. If this area below the 1952 proglacial area is capable of releasing NH_4^+ then the depth of sediment required to produce the N flux would only be 1.1m; this is likely well beyond sediment production of Forget-Me-Not Glacier and would still not account for the discrepancy between NH_4^+ and NO_3^- (Figure 3.3 and 3.4).

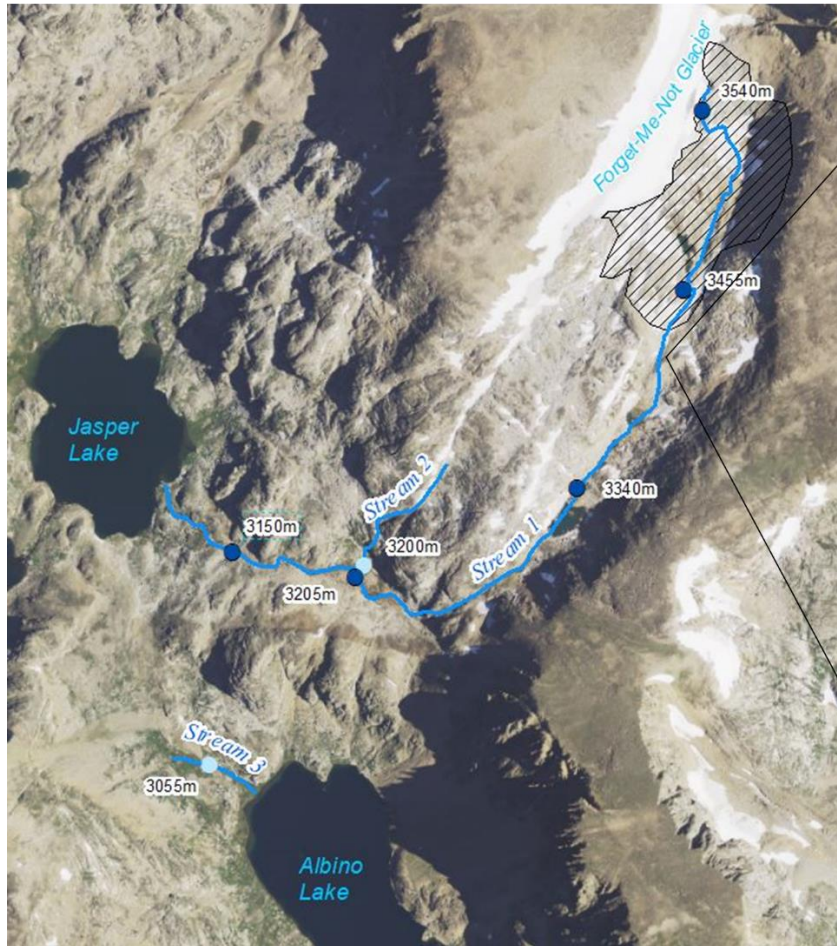


Figure 4.4 Poorly sorted sediments in the Jasper Lake catchment, where the glacially sourced stream disappears into the sediments, 50m downstream from the delineated proglacial area, maximizing water/sediment interactions. The photo was taken looking downstream to the south west.

Atmospheric

N can be deposited to the Beartooth catchments via dry or wet deposition.

Atmospheric deposition in this study was only examined via precipitation events (wet deposition), and only in the form of snow, due to the near absence of precipitation in the form of rain during field sampling visits to the catchment.

Concentrations of N measured in the in the snow pack at Clay Butte in conjunction with SNOTEL data on SWE from Beartooth Lake located approximately 8.8km to the south-south-west of the field area (Figure 2.7) were used to derive an estimate of the amount of N deposited via wet deposition in the field area. The estimated amount of N deposited through wet deposition did not provide sufficient N to account for the highest N flux estimate for the catchment (Table 3.2 and 3.7).

Results from the two component mixing model using $\Delta^{17}\text{O}$ nitrate values (following Michalski et al., 2004) show that the contribution from atmospheric input to stream water nitrate is never higher than 30% at any of the sample locations and decreases as the melt-season progresses (Figure 3.6). From DoY 229-275 the atmospheric nitrate input to stream waters does not exceed 10% based on $\Delta^{17}\text{O}$ values.

Biologic

Nitrifying bacteria are capable of converting ammonium in to nitrite (family Nitrosomonadaceae: *Nitrosospira* (also capable of complete nitrification) (Daims et al., 2015), *Nitrosovibrio*, *Nitrosomonas*) and nitrite to nitrate (family Nitrobacteraceae: *Nitrobacter*) (Koops and Pommerening-Roser, 2001). Phylotypes related to known nitrifiers were found in both catchments at all sites, but always at low abundance,

constituting less than 0.4% of total 16S rRNA gene sequences recovered at all sites. Nitrifiers were found at all sites and at higher relative abundances (Table 3.4) in the glacial catchment than compared to the nonglacial catchment.

The enrichment experiments for nitrifiers using stream sediments containing native microbes did not show an increase in oxidation of ammonium or nitrite when compared to abiotic controls (Figures 3.12 and 3.13). The enrichment culturing experiments were not performed on samples from the higher elevation sites (3340m, 3455m, and 3540m) and therefore no conclusions can be drawn relative to these sites.

Rock Analysis

XRF analysis of representative rock samples from the Jasper Lake Catchment demonstrated that the major rock type, a felsic granular gneiss, (likely trondhjemite based on high Na to Ca ratios (see Table 3.3) contained 2.78% K₂O by weight. This would result in an estimate of between 300 and 600ppm N derived from K₂O weight percent, based on estimated concentrations reported from metamorphic rocks (Bebout and Fogel, 1992).

Thin section analysis of a representative granular gneiss from the lower Jasper Lake Catchment (see Golden Rock Sample in Figure 2.2) shows many potassium bearing minerals where the potassium could act as potential replacement sites for ammonium in their crystal lattices. Potassium feldspars and mica minerals are common in the thin sections of the most representative samples of felsic granular gneiss collected from the proglacial area, making up more than 50% of the mineral area in the thin section present

in some cases. The presence of myrmekite (Kehelpannala and Ratnayake, 1999) and sericite (Maineri et al., 2003) in representative samples of the Jasper Lake Catchment indicate metasomatic conditions in the past which could have aided in the replacement of ammonium for potassium in mineral lattices.

The $\delta^{15}\text{N}$ nitrate values in the stream waters range from 1 to 10‰, and are distinct from atmospheric sources, -5 to -8‰, (Figure 3.9). Stream water $\delta^{15}\text{N}$ nitrate values are within the range of values (1 to 10.2‰) previously reported for granitic and altered granitic rocks (Holloway and Dahlgren, 2002). Given the catchment rocks have similar $\delta^{15}\text{N}$ values and mineralogy (bearing orthoclase and micas) to the rocks described by Holloway and Dahlgren (2002), then this would be consistent with a geologic source for a significant proportion of the N measured in the stream waters in the Jasper Lake Catchment. Nitrification produces more negative $\delta^{15}\text{N}$ nitrate values relative to the ammonium source (Li et al., 2019) so any nitrate sourced from bedrock is likely to have elevated $\delta^{15}\text{N}$ values relative to the stream water nitrate. Stream waters are likely still consistent with the $\delta^{15}\text{N}$ values observed in granitic rocks by Holloway and Dahlgren (2002). N_2 fixation is thought to typically change $\delta^{15}\text{N}$ values by 5‰ or more, and is a possible cause for elevated $\delta^{15}\text{N}$ (Högberg, 1997).

Historic Areal Extent of the Forget-Me-Not Glacier

Forget-Me-Not Glacier has been in retreat since at least 1952 (see Figure 4.5) and almost certainly since the Little Ice Age (LIA). The area covered by the glacier has decreased by approximately 69% from 1952-2015 and its terminus has retreated

approximately 0.5km during the same time frame. The retreat of the Forget-Me-Not Glacier is comparable to that of Castle Rock Glacier that lies approximately 9km to the northwest and has a similar elevation and aspect. Castle Rock Glacier has decreased areally by more than 90% over the same time period (1952-2015), though its terminus has retreated a comparable 0.5km.

Glacier retreat in the Beartooth Mountains over the past 70 years is also consistent with broader regional trends. The glaciers that drain the eastern side of Gannett Peak in the Wind River Range of West-Central Wyoming cover a similar elevation range as the Beartooth Mountain glaciers, and have also undergone significant retreat in the recent Holocene (since the LIA 300-100 BP) (Meier, 1951). The area covered by the greater Dinwoody Glacier has decreased by more than 30% from 1966-2014 with 1.25 km of terminus retreat since 1966. Based on Meier's analysis of Dinwoody Glacier's LIA extent and visible trim lines the areal reduction in ice cover between the LIA and 1951 was 9.5%.

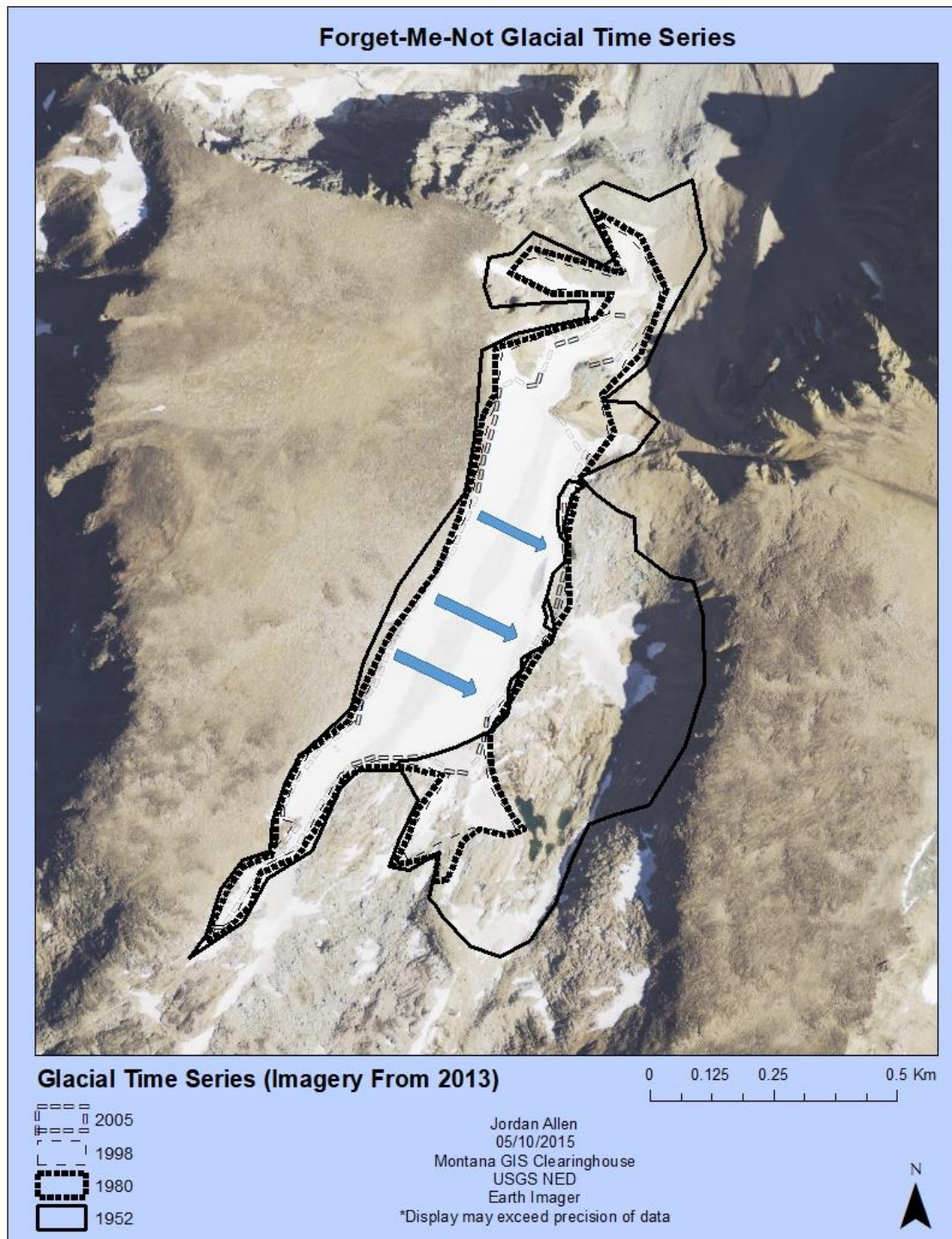


Figure 4.5 Forget-Me-Not Glacier areal extent over the past 70 years. Arrows on the glacier approximate the present day average flow direction of ice. Note: small spatial inaccuracies of boundaries may exist when viewing the images at such a large scale due to the resolution of the aerial imagery.

Field observations and imagery of scoured rock surface and deposited material indicate that the Forget-Me-Not Glacier was more extensive prior to 1952 (the onset of aerial imagery). Since these areas down valley of the 1952 ice extent show relatively fresh rock surfaces and sparse vegetation (Figure 4.6), it is reasonable to hypothesize that there was ice retreat in the early part of the 20th century and possibly prior to that also. Therefore, one can estimate a potential LIA extent for the Forget-Me-Not Glacier that extends into the small catchment that feeds the sample site at 3205m (Figure 4.6). However, confidence in the precise LIA extent of the Forget-Me-Not Glacier is low compared to the LIA extent of glaciers such as the Dinwoody Glacier in the Wind River Range due to less prevalent features that delimit the former ice margin such as large moraines and clear trim lines. However, the geomorphological data supports the notion that glacial comminution of bedrock would have extended well beyond the documented extent of the glacier in 1952. Therefore, if the release of ammonium from glacially crushed rock has been persistent since the LIA, then the area that is contributing N to the stream waters may be significantly larger than the “proglacial” area used to determine the depth of sediment needed to produce the estimated catchment N flux.

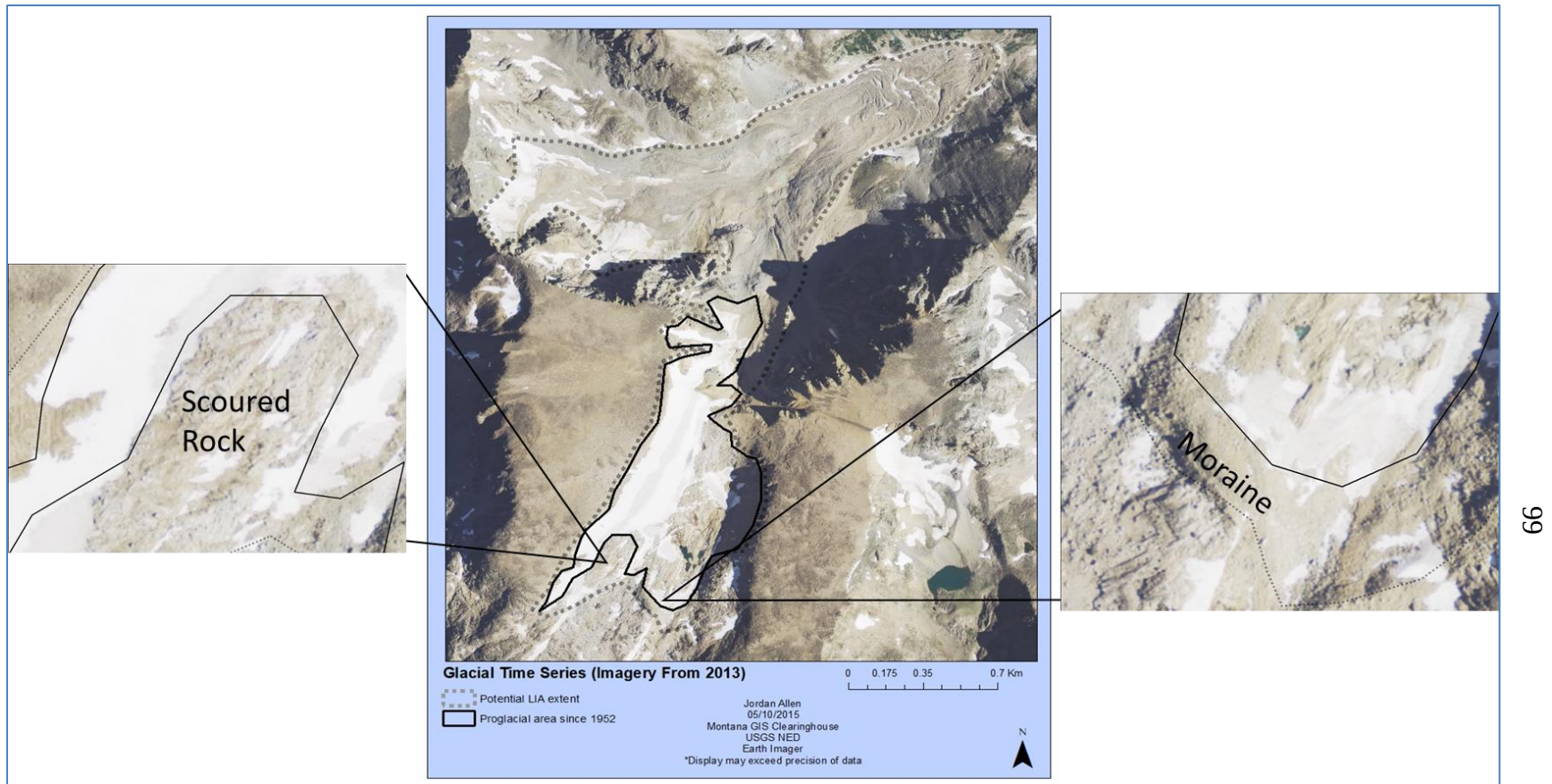


Figure 4.6 Estimated extent of the Forget-Me-Not Glacier during the LIA. Imagery of Forget-Me-Not Glacier is from 2013, and the extent of the glacier from 1952 is shown. Inset photos show glacial features that indicate a recent glacial extent beyond the current and 1952 extent.

Summary

Nitrate concentrations in the stream draining the GSF Jasper Lake Catchment, are higher when compared to the neighboring purely SF Albino Lake catchment, which is consistent with prior research (Saros et al., 2010). The relatively high concentrations of nitrate (compared to SF catchments) were measured throughout the entirety of a melt-season (DoY 189-275) and also at numerous points down stream from the source glacier, to the first major waterbody in the catchment (Figure 3.3), showing that nitrate concentrations in stream water increase as the melt-season progresses, and also increase downstream in the catchment with increasing distance from the glacial source.

Ammonium stream water concentrations were also quantified at a number of sites downstream from the glacier and also temporally over a summer melt-season. These measurements, showed high concentrations at the terminus of the glacier that generally increased as the melt-season progressed, supporting the idea that ammonium at this site is sourced from sub glacial sediment, but with generally low concentrations at all other sites (Figure 3.4). Ammonium concentrations are not high enough to account for the nitrate seen in glacial stream 1.

The estimated N flux from the atmosphere to the glacial catchment is likely inadequate to account for the N flux estimate in the glacial waters close to the inlet to Jasper Lake (Table 3.8) and this is supported by data on the isotopic composition of stream water nitrate (Figure 3.6). Abiotic weathering experiments have demonstrated that rocks from the proglacial area contain, on average, 57 μmol of N per kg. Calculations based on the N released during laboratory weathering experiments and the proglacial area

provide estimates of the sediment volume required to produce the N flux estimates in stream waters after subtracting the amount deposited from the atmosphere, and are broadly consistent with field observations (Figures 4.1 and 4.2) if sediment production is not required for continued release of N species. The $\delta^{15}\text{N}$ values of nitrate from the glacially sourced stream waters are consistent with values found in rocks of similar chemical composition (Holloway and Dahlgren, 2002).

Biotic laboratory enrichment experiments did not provide evidence for active nitrification, showing no significant decrease in ammonium or increase in nitrate. However, 16S rRNA gene sequences from putative nitrifiers were recovered at all sites sampled. Putative nitrifiers are on average an order of magnitude more abundant in the glacial catchment than the nonglacial catchment. Still, the results from the microbiological analyses in this study are inconclusive relative to active nitrification occurring in the subglacial or proglacial environments at Forget-Me-Not Glacier, as enrichments were unsuccessful in showing active nitrification using native sediments.

Future Work

While the presence of glaciers and increased N flux through a catchment (when compared to similar catchments without active glaciers) seems well established (Saros et al., 2010; Williams et al., 2007) and corroborated by this study, the timescale of this relationship is not well understood. Conflicting results of previous work (Williams et al., 2016) do not show an increase in nitrate concentrations should be examined and expanded upon to determine what factors, beyond the presence of a glacier lead, to the

increased nitrate found in the Beartooth Mountains and other environments (glaciers in Svalbard (Wynn et al., 2007) and rock glaciers in Colorado (Williams et al., 2007)). It would appear that glaciers are a necessary, but not solely fulfilling condition to yield increased nitrate in downstream waters (Williams et al., 2016); understanding what other conditions (e.g. precipitation patterns/amounts, underlying lithology) are necessary in order to yield increased nitrate in stream waters should be undertaken to more fully understand N cycling in alpine environments.

Future research should look to gain a stronger understanding of the duration of the relationship between glaciers and increased stream water nitrate. Dating of glacially scoured bedrock and glacial features (e.g. moraines) in combination with geochemical signatures (such as increased nitrate in downstream stream waters) could be used to better understand how long paraglacial processes have a significant impact on N cycling in recently deglaciated catchments. The results from this research indicate that in recently deglaciated environments, elevated nitrate levels likely persist for ~150 years (based on the elevated nitrate levels at site 3205m) but are no longer elevated on longer timescales (no elevated nitrate observed at site 3055m, which was certainly deglaciated 15 ka (Licciardi and Pierce, 2018), but exact timing of perennial ice disappearance is not known). Absolute and relative dating methods (for example, photogrammetry, radiocarbon, weathering rinds, Schmidt-hammer rebound, lichenometry, luminescence, cosmogenic exposure) could more tightly constrain the timescale of the persistence of elevated nitrate after deglaciation (Haeberli et al., 2003).

Direct measurements of N in rocks (using spectral methods, pyrolysis, Kjeldahl extracts, or sealed-tube combustion (Holloway and Dahlgren, 2002)) of different bedrock lithologies could provide additional information on the lability of the different components of N in the bedrock. Continued release of N from sediments should also be examined in abiotic weathering experiments; this could be examined using a flow-through design or varying sediment-water proportions.

The spatial relationship between nitrifiers and the increased concentration of nitrate is not well understood. Further enrichment experiments, using native waters and sediments (Boyd et al., 2011), in conjunction with laboratory bioreactor experiments (Sinha and Annachhatre, 2007) could help determine whether nitrifiers are present and where they are most abundant in the catchments. While the presence of bacterial N₂ fixers were found at all sites, further work to understand their activity and importance in the Beartooth Mountains could shed light on their potential influence on increasing nitrate concentrations. Furthermore, sampling genetic material at different times during the year could provide information on how microbial communities potentially change over the course of a melt-season in the catchments.

APPENDICES

APPENDIX A

BACTERIAL 16S rRNA GENE LIBRARY COMPOSITIONS

Table A1 Brief description and the percentage of total 16S RNA gene sequences of putative nitrifiers in the two catchments

Description	% of total 16S RNA gene sequences
<i>Nitrosomonas</i> sp. BF16c57 16S ribosomal RNA gene, partial sequence	0.364
Uncultured <i>Nitrospira</i> sp. clone LTSP_BACT_P4G19 16S ribosomal RNA gene, partial sequence; 16S-23S ribosomal RNA intergenic spacer, complete sequence; and 23S ribosomal RNA gene, partial sequence	0.170
Uncultured <i>Nitrospira</i> sp. clone sf-32 16S ribosomal RNA gene, partial sequence	0.161
<i>Nitrospira lacus</i> strain APG3, complete genome. <i>Nitrospira lacus</i> sp. nov., a psychrotolerant, ammonia-oxidizing bacterium from sandy lake sediment	0.014
Uncultured <i>Nitrospiraceae</i> bacterium clone Amb_16S_432 16S ribosomal RNA gene, partial sequence	0.014
Closest relative (99%) Uncultured <i>Nitrospira</i> sp. clone sf-32 16S ribosomal RNA gene, partial sequence	0.005
Closest relative (99%) Uncultured <i>Nitrospira</i> sp. clone LTSP_BACT_P4G19 16S ribosomal RNA gene, partial sequence; 16S-23S ribosomal RNA intergenic spacer, complete sequence; and 23S ribosomal RNA gene, partial sequence	0.002
Closest relative (93%) <i>Nitrospira</i> enrichment culture clone Ga3a 16S ribosomal RNA gene, partial sequence	0.005
Closest relative (98%) Uncultured <i>Nitrospira</i> sp. clone LTSP_BACT_P4G19 16S ribosomal RNA gene, partial sequence; 16S-23S ribosomal RNA intergenic spacer, complete sequence; and 23S ribosomal RNA gene, partial sequence	0.002
Closest relative (98%) Bacterium enrichment culture clone Anammox_26 16S ribosomal RNA gene, partial sequence	0.002
<i>Nitrospira lacus</i> strain APG3, complete genome. <i>Nitrospira lacus</i> sp. nov., a psychrotolerant, ammonia-oxidizing bacterium from sandy lake sediment	0.002
<i>Nitrospira lacus</i> strain APG3, complete genome	0.002
Closest relative (94%) <i>Nitrospira</i> sp. Enl299 16S ribosomal RNA gene, partial sequence	0.002
Closest relative (99%) <i>Nitrosomonas aestuarii</i> strain Nm36 16S ribosomal RNA, partial sequence	0.002
<i>Nitrospira briensis</i> C-128, complete genome.	0.002

Closest relative (98%) <i>Nitrosospira briensis</i> C-128, complete genome	0.002
Closest relative (99%) Uncultured <i>Nitrospira</i> sp. partial 16S rRNA gene, clone JG36-GS-2	0.002
Closest relative (94%) <i>Nitrosomonas communis</i> strain Nm2, complete genome	0.002
Closest relative (98%) <i>Nitrosospira</i> sp. III7 16S ribosomal RNA gene, partial sequence	0.002
Closest relative (98%) Bacterium enrichment culture clone Anammox_26 16S ribosomal RNA gene, partial sequence	0.002
Closest relative (99%) Uncultured <i>Nitrospira</i> sp. clone sf-32 16S ribosomal RNA gene, partial sequence	0.002
Closest relative (98%) <i>Nitrosospira</i> sp. Enl299 16S ribosomal RNA gene, partial sequence	0.002
Closest relative (98%) <i>Nitrosomonas</i> sp. BF16c57 16S ribosomal RNA gene, partial sequence	0.002

Table A2 Most closely related cultured representative for the 23 most abundant phylotypes, based on partial 16S rRNA gene sequence, with short descriptions and their percentage of total 16S rRNA gene sequences at each site. Top 23 most abundant phylotypes are shown because of three species associated with human presence (*Staphylococcus epidermidis* strain FDAARGOS, *Cutibacterium acnes* JK18.2, and *Streptococcus pneumoniae* strain 11A). These three human associated species are included for completeness, but the table is completed to show the top 20 most abundant phylotypes if human associated species are not considered, as they were only abundant at the lowest (3055m) site.

3150m % Copies/Li ter 4.09E+07 Stream 1	3340m % Copies/Li ter 2.83E+07 Stream 1	3455m % Copies/Li ter 2.39E+08 Stream 1	3540m % Copies/Li ter 2.47E+08 Stream 1	3205m% Copies/Li ter 3.53E+07 Stream 2	3055m % Copies/Li ter 4.06E+06 Stream 3	Description
11.3	8.2	7.7	7.0	4.9	2.1	<i>Massilia psychrophilum</i> sp. nov., a novel purple-pigmented bacterium isolated from Tianshan glacier, Xinjiang Municipality, China.
4.4	23.2	5.9	0.2	0.7	0.2	<i>Flavobacterium</i> sp. strain RB1R5 16S ribosomal RNA gene, partial sequence (Bernardet et al., 1996))
3.5	4.2	17.3	7.0	0.6	1.6	<i>Polaromonas</i> sp. strain W13N 16S ribosomal RNA gene, partial sequence

						(Darcy et al., 2011)
4.6	1.7	3.6	2.4	5.1	0.8	<i>Massilia</i> sp. 5S-42 gene for 16S ribosomal RNA, partial sequence (Zhang et al., 2006)
0.2	1.0	0.8	15.0	0.1	0.3	<i>Alkanindiges illinoisensis</i> strain HYN0046 16S ribosomal RNA gene, partial sequence alkane-degrading bacterium (Bogan et al., 2003)
0.4	0.4	8.4	4.3	0.1	0.6	Closest relative (98%) <i>Ferruginibacter</i> sp. strain HRS2-29 16S ribosomal RNA gene, partial sequence.
1.0	1.0	3.3	3.2	1.4	2.3	<i>Leptothrix</i> sp. strain BDP18 16S ribosomal RNA gene, partial sequence. (Ghosh et al., 2018)
0.8	0.8	0.6	3.4	6.1	0.2	<i>Burkholderia</i> sp. strain

						KBL32 16S ribosomal RNA gene, partial sequence (Coeyne and Vandamme, 2003)
0.1	0.1	0.0	0.0	1.0	9.6	<i>Staphylococcus epidermidis</i> strain FDAARGOS_161 chromosome, complete genome (Wieser and Busse, 2000)
0.4	3.5	1.0	4.9	0.0	0.0	Closest relative (98%) Uncultured <i>Candidatus Saccharibacteria</i> bacterium gene for 16S ribosomal RNA, partial sequence, clone: MSB-28.
0.6	1.2	5.4	2.3	0.0	0.5	Closest relative (99%) <i>Ferruginibacter paludis</i> strain HME8881 16S ribosomal RNA, partial sequence. (Kang et al., 2015)

1.4	0.3	0.3	0.2	4.2	3.4	Closest relative (99%) <i>Rubrivivax gelatinosus</i> strain TN414 16S ribosomal RNA gene, partial sequence.
1.0	1.8	2.2	4.4	0.1	0.0	<i>Mucilaginibacter rigui</i> strain cHCR18aj 16S ribosomal RNA gene, partial sequence Microbial communities associated with cryoconite hole water
5.4	0.3	0.1	0.0	3.3	0.0	Closest relative (98%) <i>Sediminibacterium</i> sp. MIC4028 16S ribosomal RNA gene, partial sequence.
2.1	2.0	2.0	1.9	0.3	0.7	<i>Arcicella aquatica</i> strain TP-Snow-C70 16S ribosomal RNA gene, partial sequence.
0.1	0.0	0.0	0.0	0.5	6.9	<i>Cutibacterium acnes</i> JK18.2 gene for 16S

						ribosomal RNA, partial sequence
2.1	1.5	2.6	0.5	0.4	0.0	<i>Rhodoferrax ferrireducens</i> T118, complete genome
0.0	0.0	0.0	0.0	0.3	6.7	<i>Streptococcus pneumoniae</i> strain 11A chromosome, complete genome
3.4	1.1	1.5	0.0	0.1	0.0	<i>Alcaligenes</i> sp. 92 16S ribosomal RNA gene, partial sequence, soil microbe, <i>Alcaligenes</i> is a genus of Gram-negative, aerobic, rod-shaped bacteria. Additionally, some strains of <i>Alcaligenes</i> are capable of anaerobic respiration, but they must be in the presence of nitrate or nitrite
1.4	0.2	0.2	0.0	3.8	0.5	<i>Flavobacterium</i> sp. strain k_lcl1A 16S

						ribosomal RNA gene, partial sequence
5.3	0.3	0.2	0.0	0.1	0.0	<i>Flavobacteriu m sp.</i> HME6133 16S ribosomal RNA gene, partial sequence (Bernardet et al., 1996)
0.1	0.3	0.2	4.8	0.0	0.0	<i>Flavobacteriu m sp. strain k_lc11A</i> 16S ribosomal RNA gene, partial sequence
0.5	1.2	1.5	0.7	0.9	0.4	<i>Burkholderiale s bacterium</i> W35 gene for 16S ribosomal RNA, (Hirose et al., 2016)

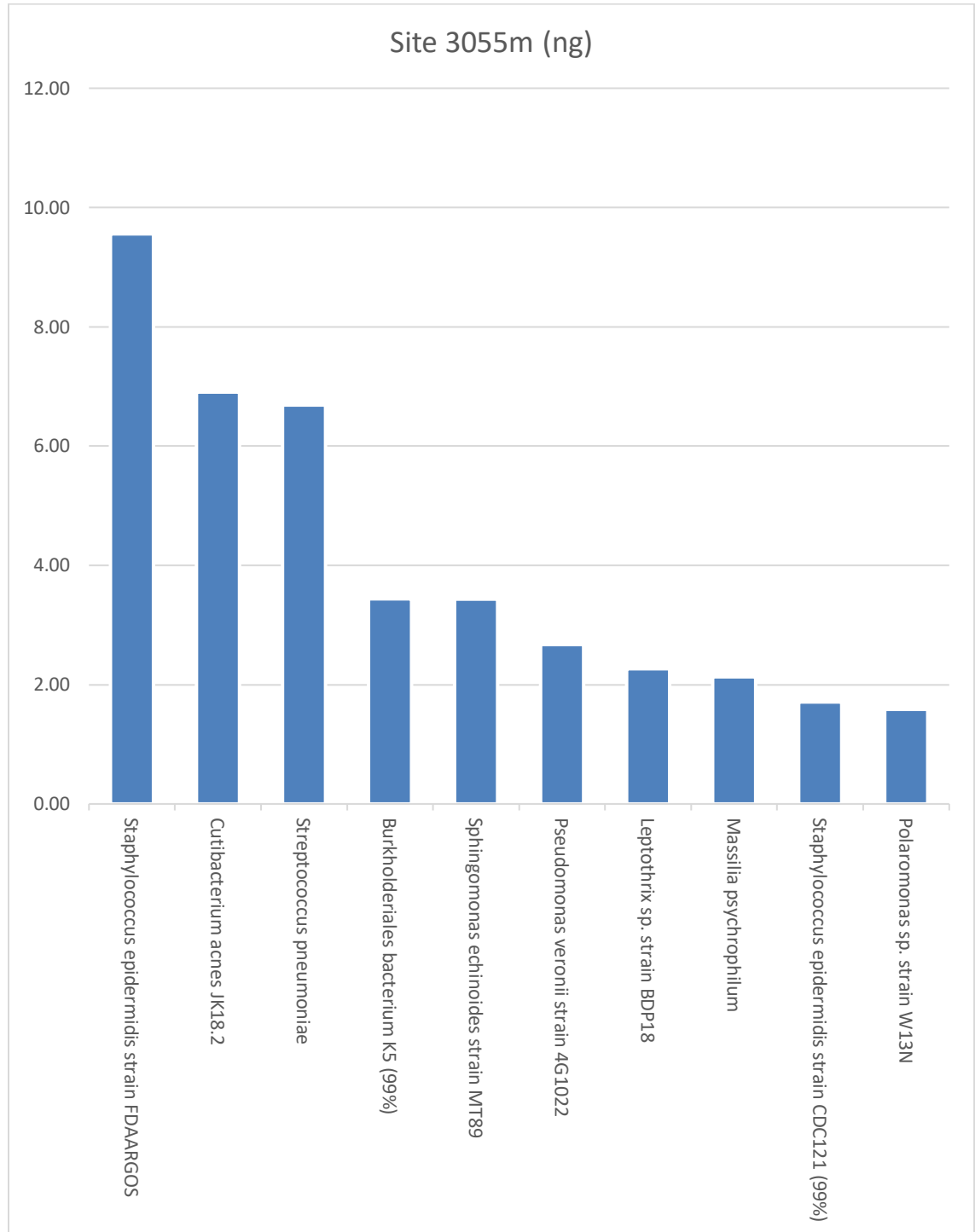


Figure A1 The top ten phylotypes by abundance, based on partial sequencing of the 16S rRNA gene (1100-1492 base pairs) is shown for Site 3055m (ng). “NG” refers to sites that are not currently sourced by glacial ice. The closest cultured representative for each phylotype in the NCBI data base is noted, if the sequenced section is not 100% match, the percent match is noted.

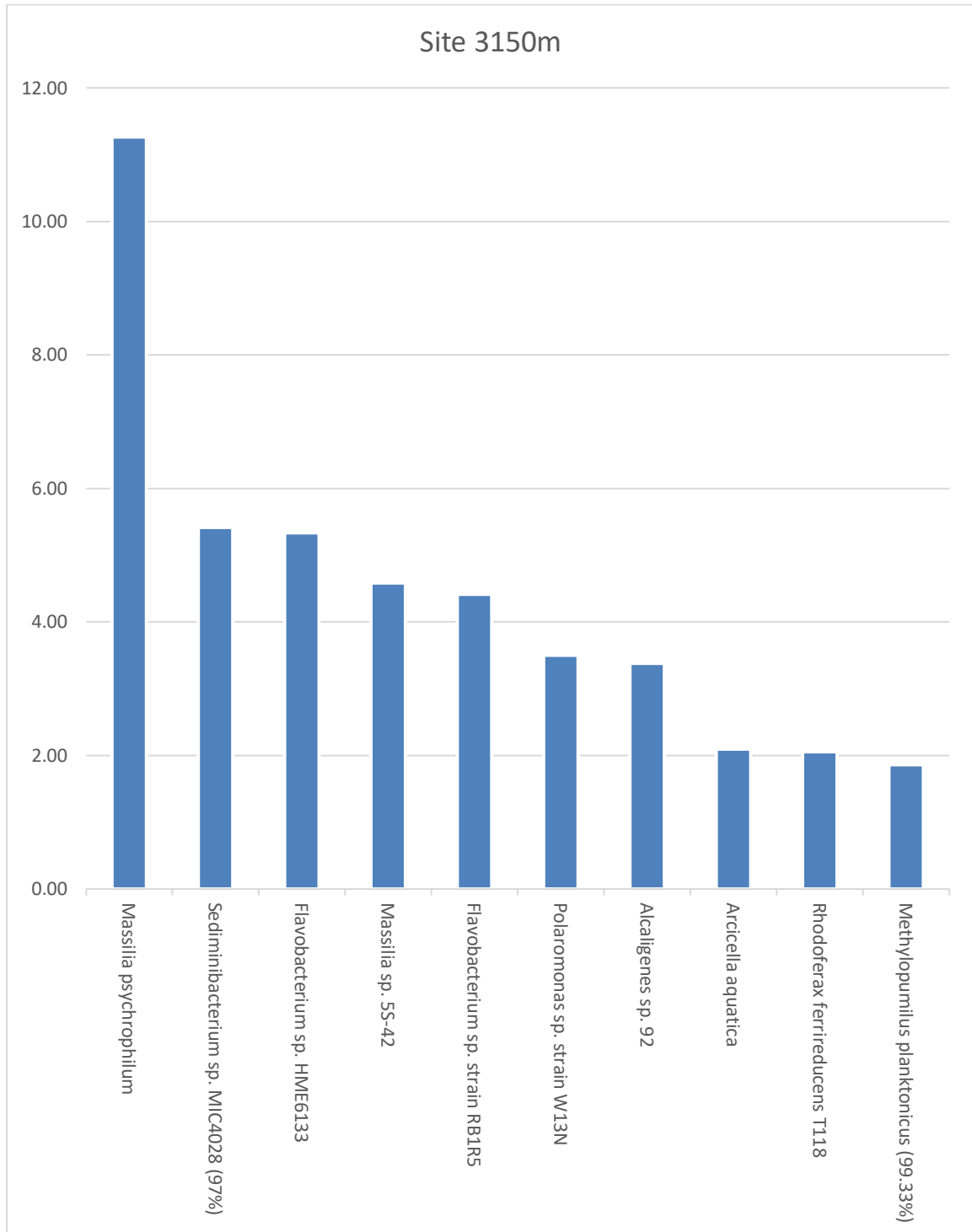


Figure A2 The top ten phylotypes by abundance, based on partial sequencing of the 16S rRNA gene (1100-1492 base pairs) is shown for Site 3150m. The closest cultured representative for each phylotype in the NCBI data base is noted if the sequenced section is not 100% match, the percent match is noted.

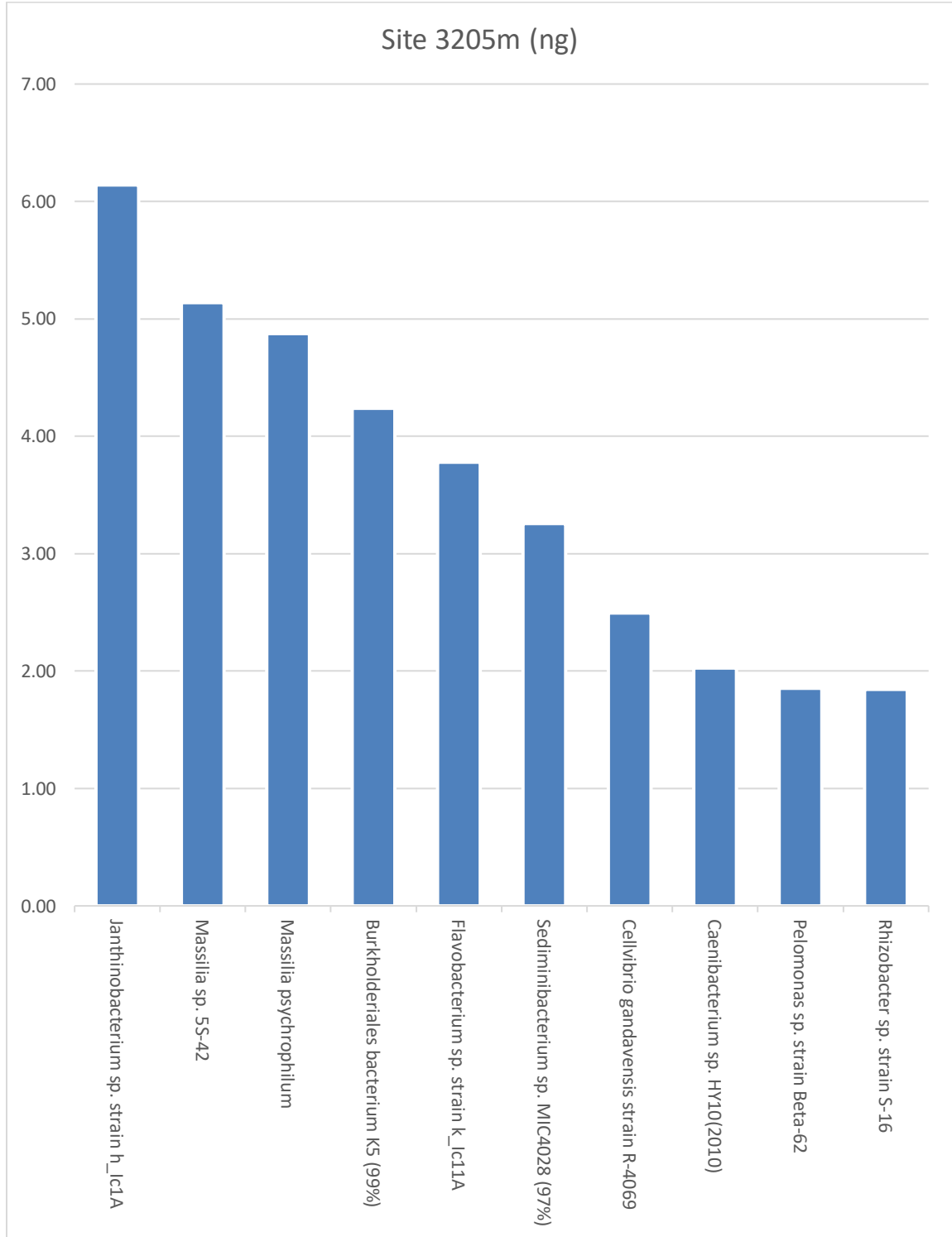


Figure A3 The top ten phylotypes by abundance, based on partial sequencing of the 16S rRNA gene (1100-1492 base pairs) is shown for Site 3205m (ng). The closest cultured representative for each phylotype in the NCBI data base is noted, if the sequenced section is not 100% match, the percent match is noted.

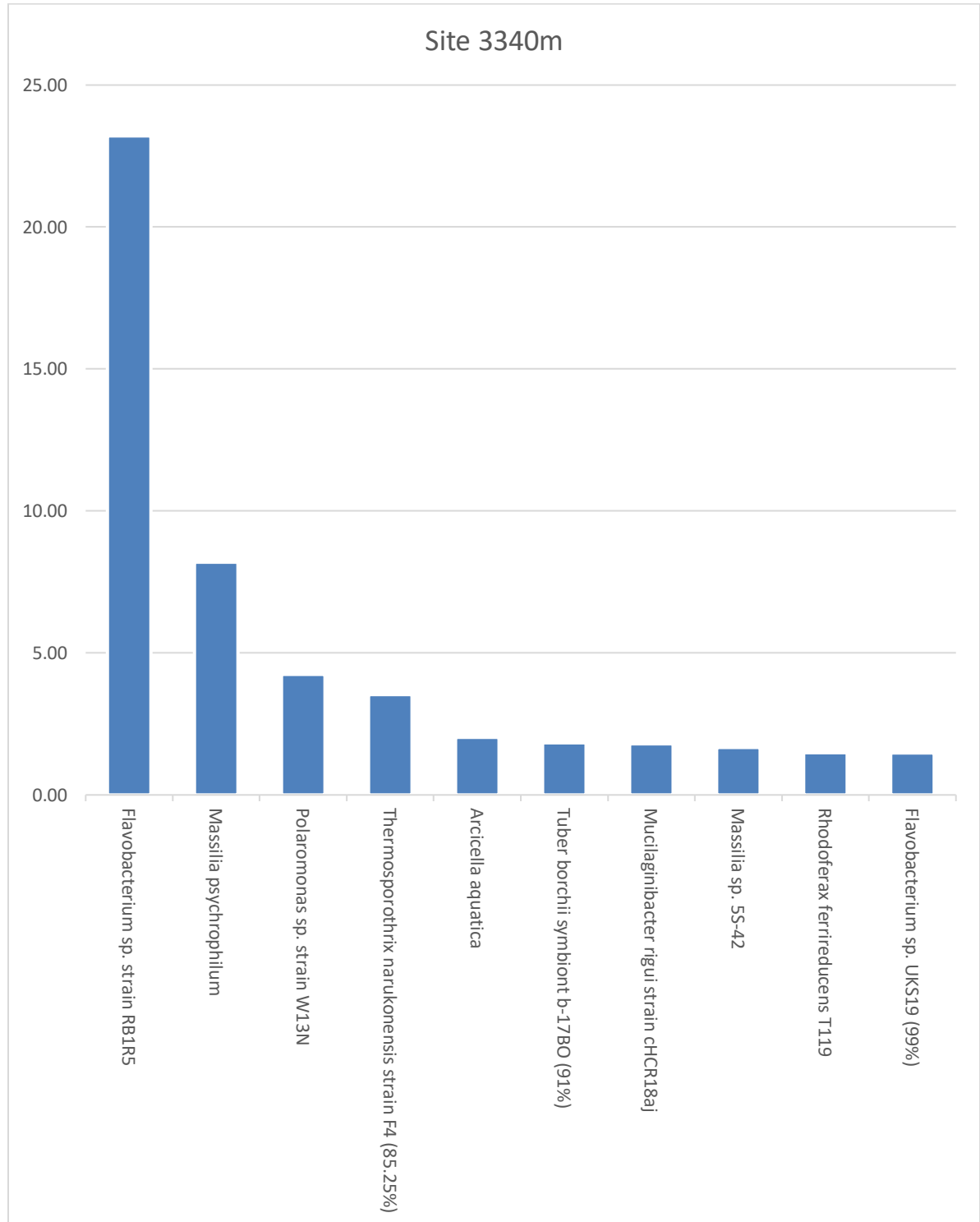


Figure A4 The top ten phylotypes by abundance, based on partial sequencing of the 16S rRNA gene (1100-1492 base pairs) is shown for Site 3340m. The closest cultured representative for each phylotype in the NCBI data base is noted, if the sequenced section is not 100% match, the percent match is noted.

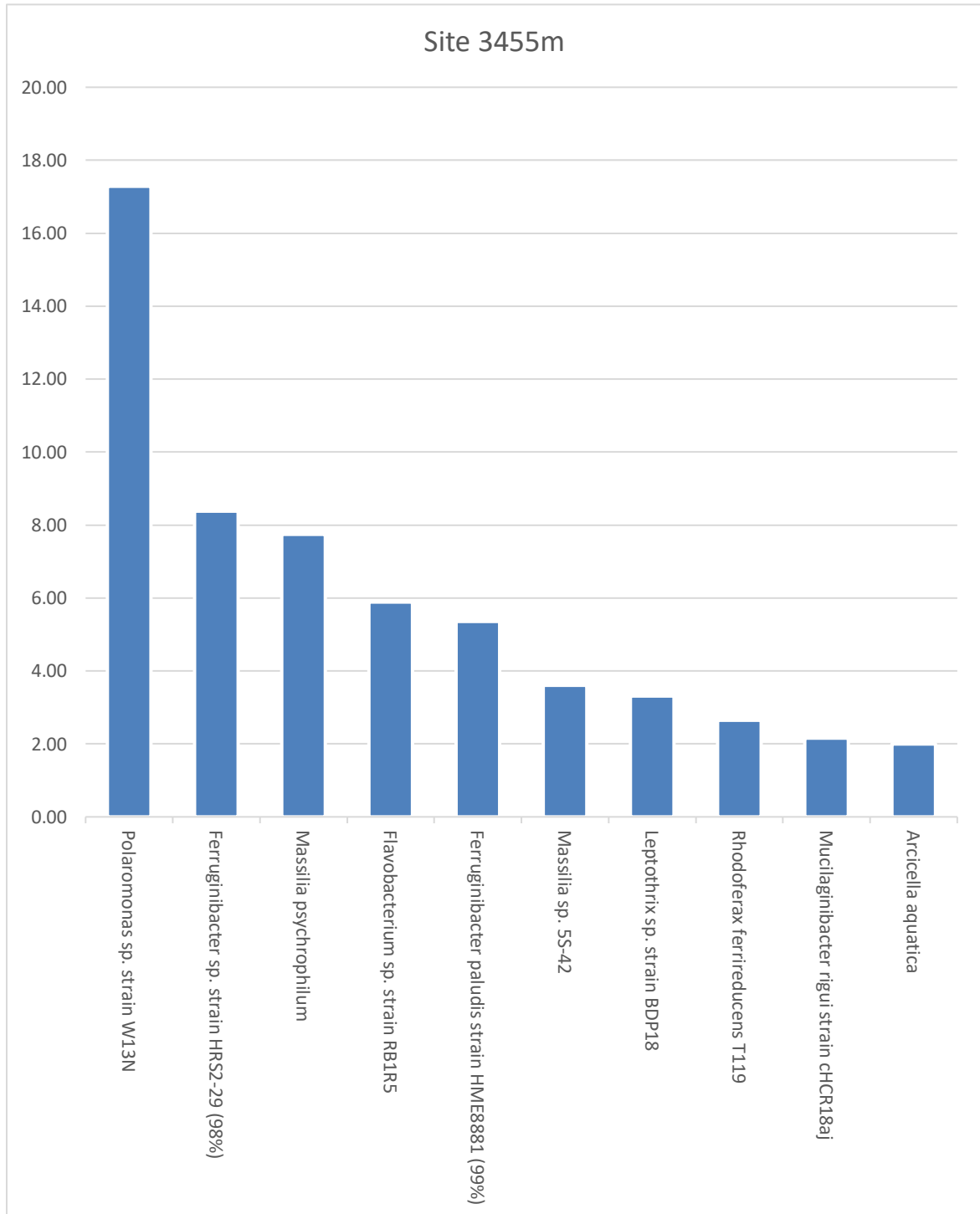


Figure A5 The top ten phylotypes by abundance, based on partial sequencing of the 16S rRNA gene (1100-1492 base pairs) is shown for Site 3455m. The closest cultured representative for each phylotype in the NCBI data base is noted, if the sequenced section is not 100% match, the percent match is noted.

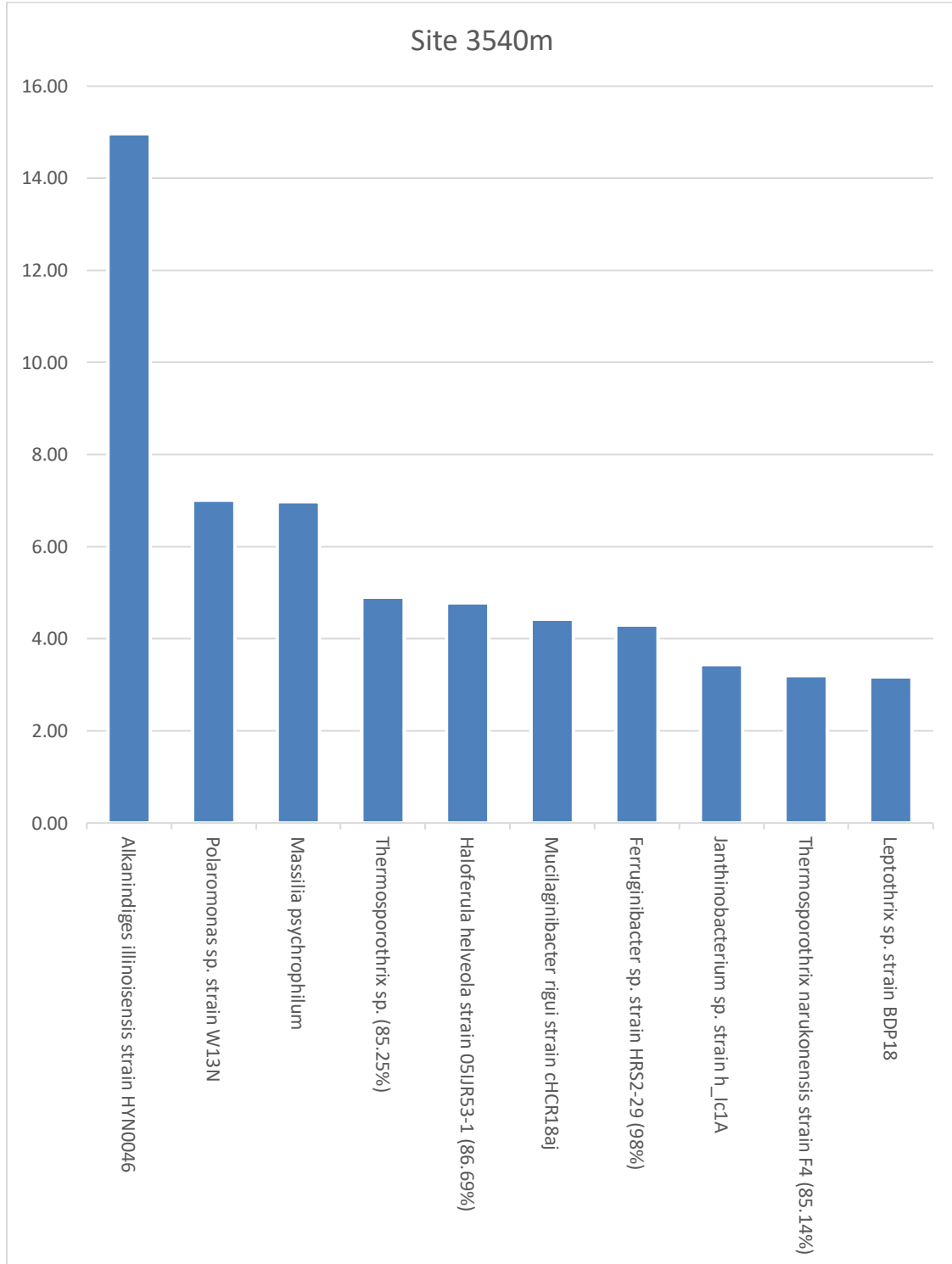


Figure A6 The top ten phylotypes by abundance, based on partial sequencing of the 16S rRNA gene (1100-1492 base pairs) is shown for Site 3540m. The closest cultured representative for each phylotype in the NCBI data base is noted, if the sequenced section is not 100% match, the percent match is noted.

APPENDIX B

MEDIA USED TO ENRICH NITRIFIERS

Table B1 The chemical constituents for the ammonium oxidizer enrichment media

Media for ammonium oxidizer enrichments	
Recipe for 1L of Media	
Preparation of 10x major elements	
Elements	Mass
MgSO ₄ *(7H ₂ O)	2g
(NH ₄) ₂ SO ₄	1.3g
K ₂ HPO ₄	0.9g
CaCl ₂ *(2H ₂ O)	0.2g
Preparation of 1000X minor elements	
Elements	Mass
MnCl ₂ *(6H ₂ O)	0.216g
NaMoO ₄ *(2H ₂ O)	0.1g
CuSO ₄ *(5H ₂ O)	0.02g
CoCl ₂ *(6H ₂ O)	0.002g
ZnSO ₄ *(7H ₂ O)	0.1g
Preparation of 10x chelated iron	
Elements	Mass
FeSO ₄ *(7H ₂ O)	0.278g
EDTA	0.3725g

Ammonium Oxidizer Media Procedure

Add components to DIW and bring volume to 1.0L. Mix thoroughly. Distribute into autoclaved (at 121°C for 15 minutes) flasks. Autoclave for 15 min at 15 psi pressure– 121°C. Media pH was 7.5.

Table B2 The chemical constituents for the nitrite oxidizer enrichment media

Nitrite Oxidizer Media	
Recipe for 1L of Media	
Compound	
NaCl	0.5 g
KH ₂ PO ₄	0.15 g
MgCl ₂ 6H ₂ O	0.05 g
FeSO ₄ 7H ₂ O	973.0 µg
H ₃ BO ₃	49.4 µg
ZnSO ₄ 7H ₂ O	43.1 µg
(NH ₄) ₆ Mo ₇ O ₂₄ 4H ₂ O	37.1 µg
MnSO ₄ H ₂ O	33.8 µg
CuSO ₄ 5H ₂ O	25.0 µg
NaNO ₂	0.5 g
CaCO ₃	0.007 g

Nitrite Oxidizer Media Procedure

- 1) Autoclave bottles at 121°C for 15 minutes. Rinse out a flask (2 times the volume of the amount of media being created) and bottles and caps with DIW.
- 2) Add 10 mL of 10 X NaCl, KH₂PO₄, and MgCl₂ stock to a 2 L flask.
- 3) 970 mL of DIW was added to flask to bring volume up to 1 L of media.
- 4) Add stir bar that was rinsed with DIW to each flask and double foil cap and autoclave along with bottles.
- 5) Add 10 mL of filter sterilized 100 X Trace Metal solutions to 1 L of media.
- 6) Approximately 0.007 g CaCO₃ added to 1 L of media and dissolved on stir plate.
- 7) Approximately 0.5 g of NaNO₂ added to 1 L of media and dissolved on stir plate.
- 8) Flame sterilizes the lip of the flask and pour media into the autoclaved bottle.
- 9) Adjust media to approximately 8.5 pH using autoclaved or filter sterilized NaOH.

APPENDIX C

THIN SECTIONS

Beartooth Mountains, Forget-Me-Not Glacier**Proglacial Area 2016****Sample: 1**

High grade amphibolite

Main Stage Metamorphic Minerals:

Amphibole (Hornblende): 45%

Plagioclase: 5%

Sanidine: 30%

Opaque: 8%

Quartz: 2%

Sericite: 10%

Accessory Minerals:

White mica: <1%

Megascopic Description:

A mostly dark rock with grains 3-5mm in size, no visible size difference in hand sample exists in hand sample. White crystals make up a small proportion (~ 5%) appear to be plagioclase based upon crystal habit. There are grey-green crystals that appear to be quartz. The majority of dark crystals have a strong cleavage and appear to be mostly biotite. No orientation of grains can be seen in hand sample

Textural Description:

No preferred crystal orientation is apparent.

Hornblende crystals are pleochroic, ranging from green to brown, and show strong cleavage. Crystals range from 0.2 to 1mm in size.

Plagioclase is rare and less than .5mm in size.

Sanidine show typical polysynthetic twinning and range in size from .5 to 1.5mm in size. Crystals commonly have undergone alteration to sericite.

Opagues range from <0.1 to 0.5mm. Some show cubic crystal habit, while others appear to be secondary, without a crystal form.

Quartz often shows undulose extinction. Crystals range from 0.1 to 0.75mm in size.

Origin and History:

Undulose extinction of quartz indicates plastic deformation of the quartz, showing that the quartz is likely primary. Sericite may indicate metasomatic conditions.



K-spar = Sericite + Quartz

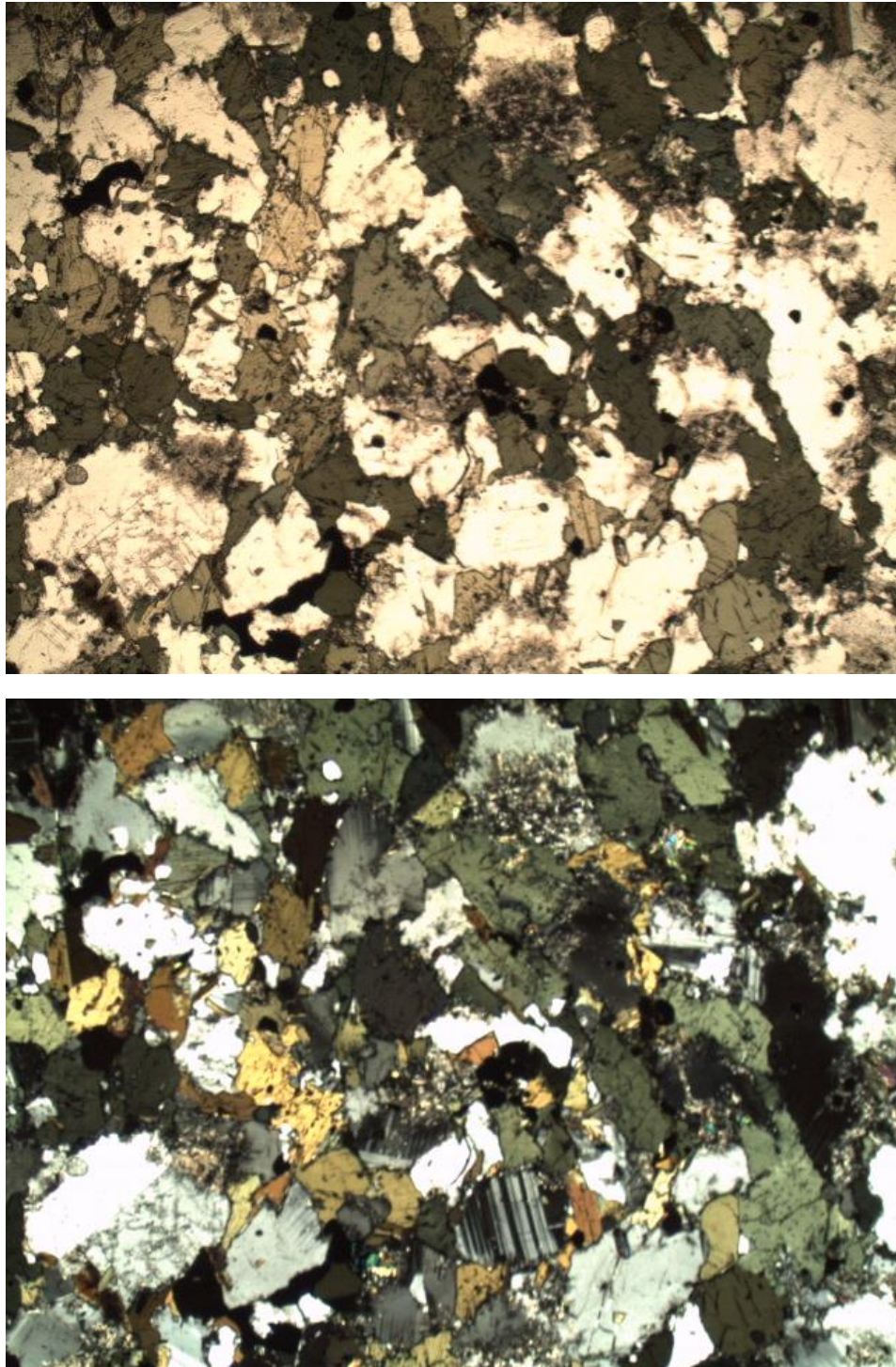


Figure C1 Proglacial Sample 1 a) in plain polarized light. The field of view is 8mm. The green-brown minerals are all amphiboles exhibiting strong pleochroism. The white minerals are mainly sanidine with small amounts of quartz and other feldspars. b) with cross polarization.

Beartooth Mountains, Forget-Me-Not Glacier**Proglacial Area 2016****Sample: 2****Main Stage Metamorphic Minerals:**

Quartz: 45%

Potassium Feldspar: 50%

Biotite: 5%

Accessory Minerals:

Muscovite: <1%

Opaques: <1%

Sericite: <1%

Megascopic Description:

White-pink rock dominated by quartz and potassium feldspar crystals up 1.5cm in size. Some small (<2mm) darker crystals can be seen as well and appear to be biotite.

Textural Description:

No preferred crystal orientation is apparent.

Quartz crystals do not exhibit undulose extinction and range in crystal size from <0.1 to 10mm in size. Quartz is sometimes intermingled with sericite (<0.1mm).

Potassium feldspar is present in the form of microcline often exhibiting tartan twinning. Crystals range from <0.1 to 6mm in size.

Biotite is present ranging in size from <0.1 to 2mm in size.

Opaques are rare and range up to 1mm in size.

Origin and History:

K-spar = Sericite + Quartz

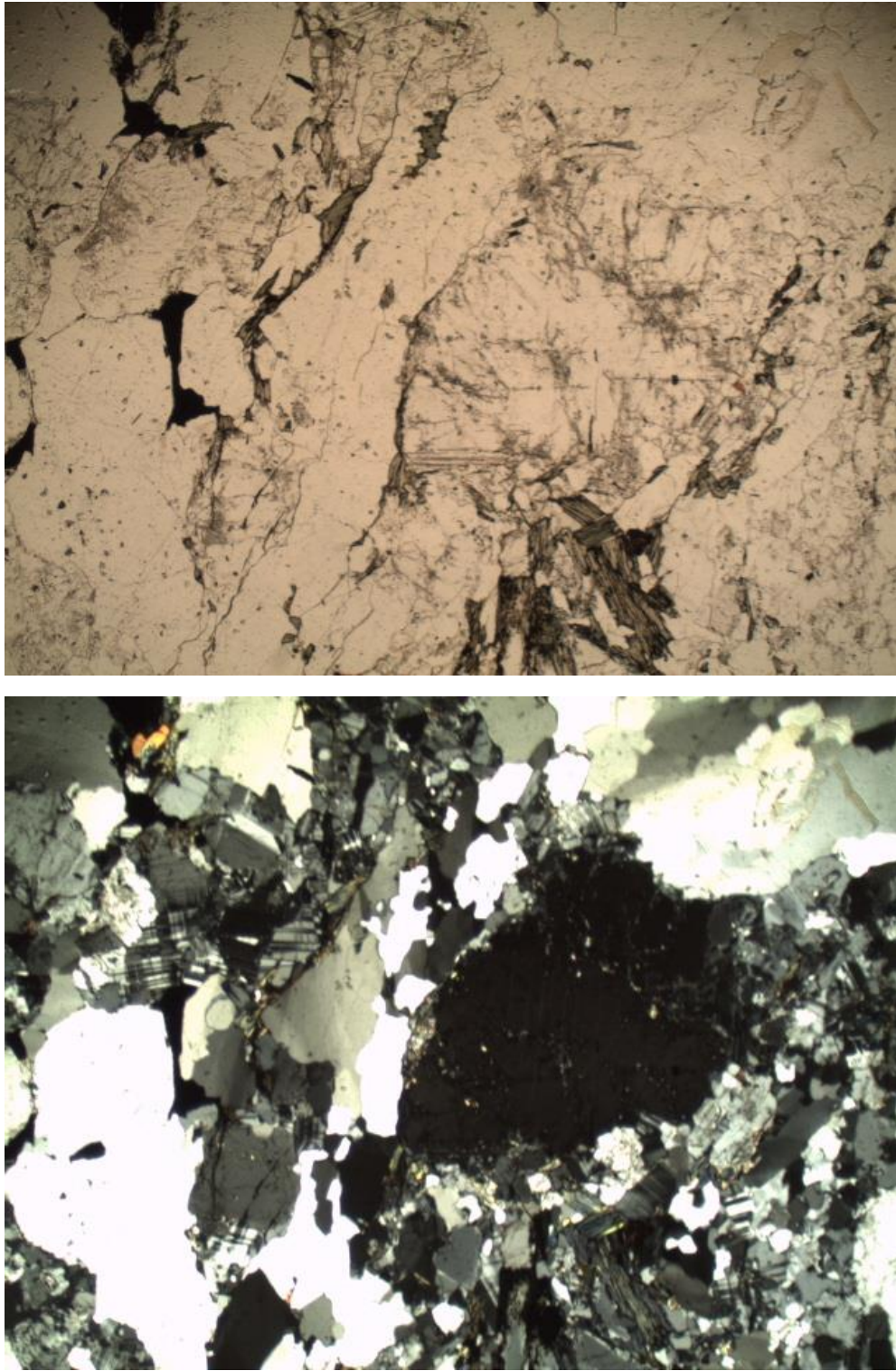


Figure C2 Proglacial sample 2 a) in plain polarized light. b) with cross polarization. Field of view is 8mm.

Beartooth Mountains, Forget-Me-Not Glacier**Proglacial Area 2016****Sample: 3****Main Stage Metamorphic Minerals:**

Quartz: 55%

Potassium feldspar: 30%

Biotite: 10%

Sericite: 5%

Accessory Minerals:

White mica: <1%

Opaques: <1%

Zircon: <1%

Megascopic Description:

White-pink-grey-black rock with large crystals of quartz and potassium feldspar up to 1.5cm in size. Grey-black components seem to be mainly biotite crystals <1mm in size.

Textural Description:

Quartz often exhibits undulose extinction. Crystals range from 0.1 to 4mm.

Potassium feldspar often shows tartan twinning (indicating microcline). Crystals range from 0.5 to 10mm. Some alteration to sericite can be seen.

Biotite is present containing crystals from <0.1 to 1mm in size. Zircons can occasionally be seen as well as their alteration halos imbedded within the biotite.

Very small quantities of white mica can be seen that are all <0.5mm in size.

Opaques are typically <1mm in size.

Origin and History:

K-spar = Sericite + Quartz

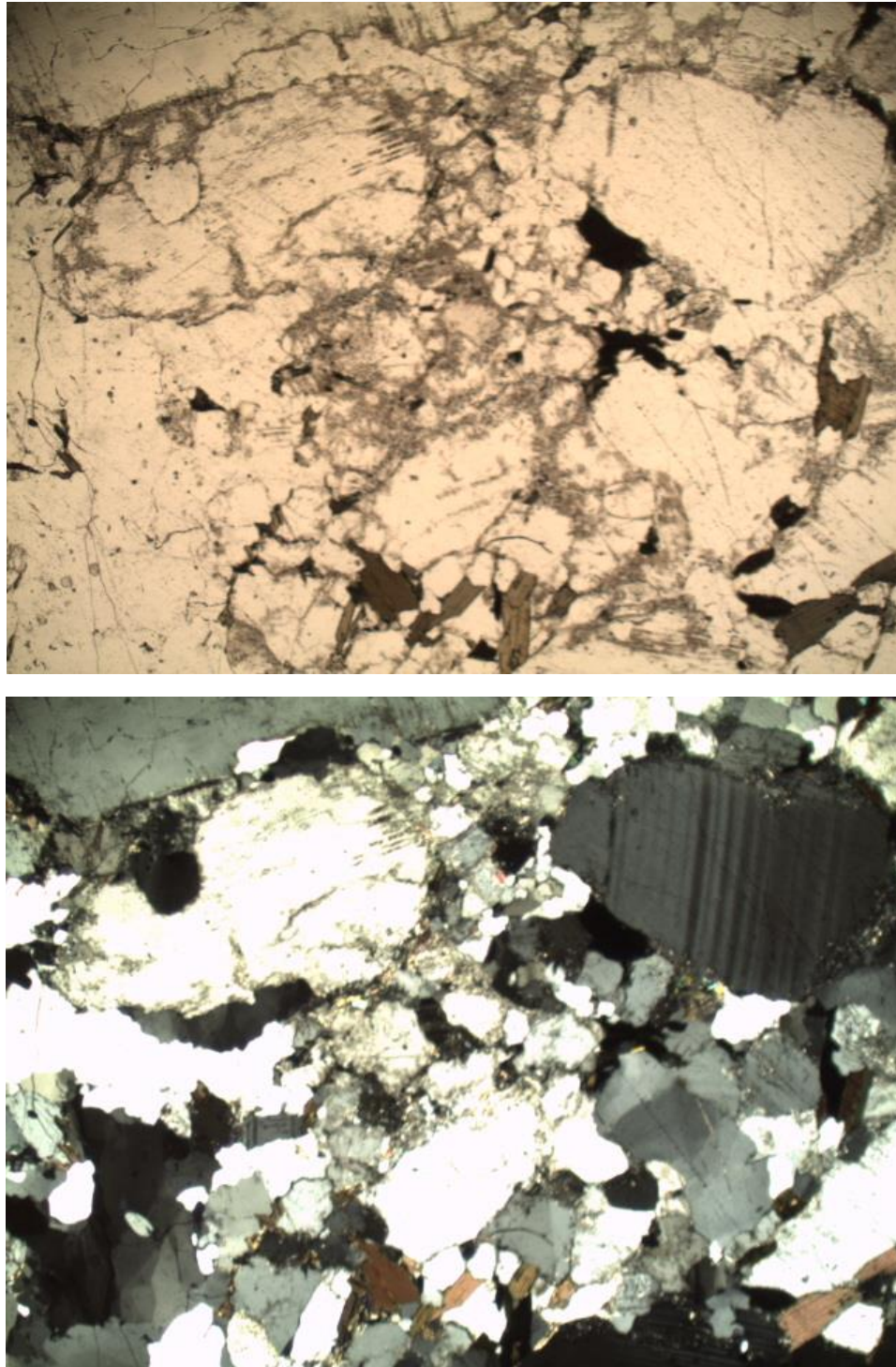


Figure C3 Proglacial Sample 3 a) in plain polarized light. b) with cross polarization. The field of view is 8mm.

Sample 4 2015**Location: Beartooth Mountains****Golden Lake****Main Stage Metamorphic Minerals**

Quartz: 28%

Plagioclase: 10%

Microcline: 30%

Orthoclase: 27%

Biotite: 2%

Hornblende: 2%

Accessory Minerals

Zircon: <1%

Muscovite: <1%

Clinopyroxene: <1%

Myrmekite: <1%

Opaque: <1%

Biotite alteration (oxides, radiation halos, others): 1%

Name: Granular Gneiss**Megascopic Description:**

Granular, un-oriented, pink-white-orange rock with large (1.5 cm) quartz, (1 cm) potassium feldspar, and (1 cm) plagioclase crystals. Much smaller (<0.1 cm) darker minerals (dark green-black) are seemingly randomly scattered and un-oriented; some of these can be identified as amphiboles with a hand lens.

Textural Description:

Quartz often exhibits mottled extinction. Quartz crystals range from .01-3.5mm in size.

Opaques are rare and never reach larger than .5 mm in length. Crystal habit is rare

Plagioclase is less common than the K-feldspars but can be large in size. Crystals range from .1-4mm

Microcline is present and in large quantities, typically .5-7 mm in size. The microcline exhibits typical tartan twinning. Because the grid twinning is an inversion texture, it shows that the original feldspar was once monoclinic.
([http://minerva.union.edu/hollochk/c_petrology/ig_minerals.htm#Potassium feldspars](http://minerva.union.edu/hollochk/c_petrology/ig_minerals.htm#Potassium%20feldspars))

Orthoclase seems to be present as well. The crystals range from .5-3mm in size; this makes sense if the slower cooling crystals became microcline with tartan twinning and were able to reach larger sizes due to their slower cooling.

Biotite is present and, along with hornblende, accounts for nearly all of the mafic minerals present in the rock. Crystals are un-oriented and reach up to .8 mm in length. Biotite appears to alter to

Hornblende is also present and is typically never larger than .7 mm.

Zircon is present as an accessory mineral in some biotites and is more easily seen by radiation halos in the biotites.

A few minor occurrences of muscovite were also seen but in very small quantities. The crystals never reached larger than .2 mm.

Clinopyroxene was seen in very small amounts and reached up to .1 mm in length.

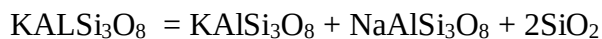
What appeared to be secondary oxides would also fill cracks in or around other crystals.

Myrmekite is also seen, with quartz growths never being larger than .05mm

Origin and History:

Slow cooling of orthoclase led to the development of microcline.

Myrmekite indicates metasomatic conditions of this rock.



H-T K-feldspar myrmekite

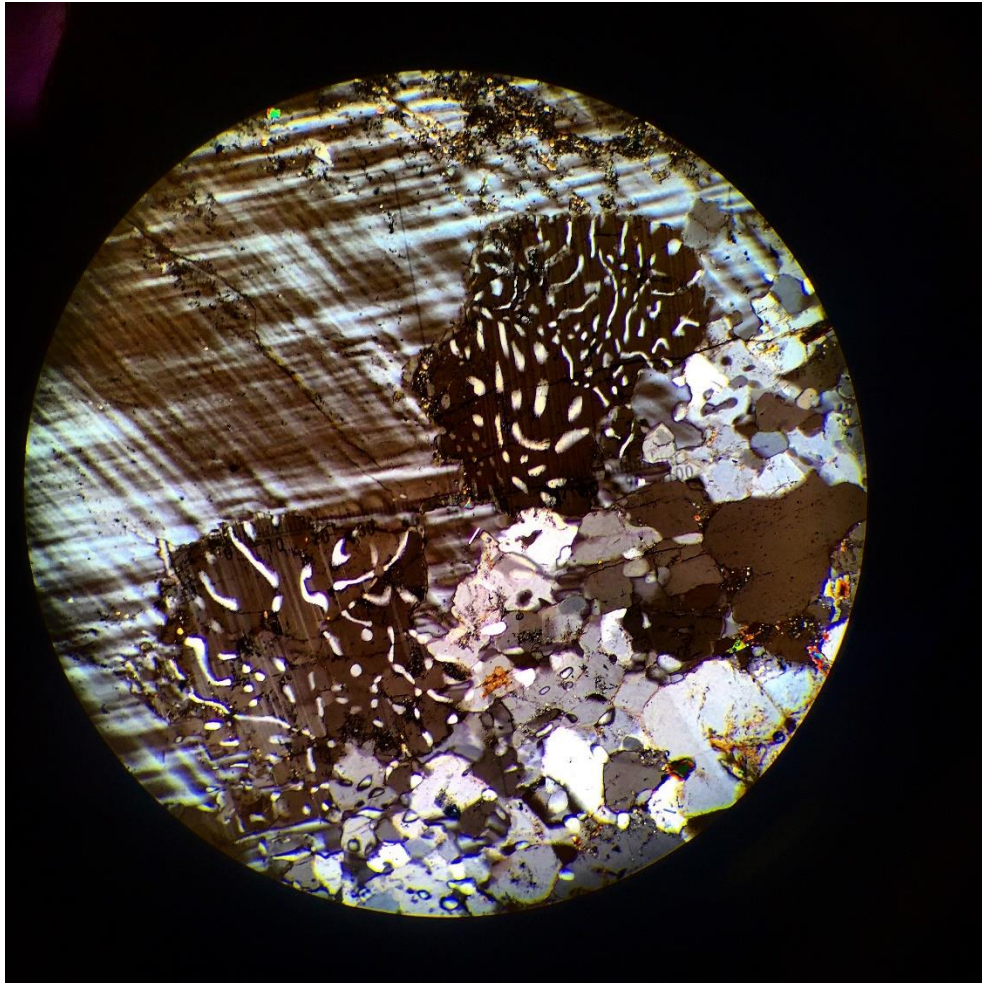


Figure C4: 5XFOV=4mm, Myrmekite visible in both the upper right and bottom left of the image.

APPENDIX D

WATER MEASUREMENTS

Table D1 EC, and temperature measurements of the stream waters at the sampling sites in the Jasper and Albino Catchments.

Site Elevation (m)	EC ($\mu\text{S}/\text{cm}$)	Temp $^{\circ}\text{C}$	Time	DoY	Notes
3055	5.5	10.5	16:30	189	
3055	6.4	12.7	17:00	201	
3055	7.0	14.6	16:00	215	
3055	6.8	12.4	17:30	229	
3055	7.0	11.0	12:30	238	Precipitation night before
3055	7.5	9.0	12:00	253	Precipitation night before
3055	6.6	9.7	15:30	275	
3150	4.7	5.8	12:45	189	
3150	5.2	9.2	16:30	200	
3150	4.8	11.0	17:00	214	
3150	7.0	9.9	17:00	228	
3150	8.2	1.2	08:45	238	Precipitation night before
3150	10.8	2.1	10:30	253	Precipitation night before
3150	12.9	7.9	15:50	274	
3200	4.4	6.1	14:30	189	
3200	4.9	7.2	17:45	200	
3200	4.4	8.6	18:15	214	
3200	5.1	10.5	14:15	229	
3200	7.7	4.8	10:00	238	Precipitation night before
3200	10.5	4.4	16:20	252	Light snow
3200	12.6	7.5	17:30	274	
3340	3.7	2.0	10:00	201	
3340	3.9	3.3	09:00	215	
3340	4.8	7.8	13:00	229	Smoky
3340	7.8	3	08:45	239	
3340	10.6	2.7	11:45	252	
3340	10.0	3.2	10:00	275	
3455	2.7	1.0	11:00	201	
3455	2.5	4.4	10:30	215	
3455	3.4	7.1	11:30	229	
3455	6.2	5.4	10:45	239	
3455	7.7	4.9	13:00	252	
3455	7.4	5.9	11:30	275	
3540	1.7	3.9	12:00	201	
3540	1.5	2.1	11:30	215	
3540	3.0	5.2	10:30	229	

3540	3.1	4.6	11:30	239	filtered at 3455 because of weather
3540	4.8	1.8	14:30	252	
3540	3.1	2.5	12:30	275	
3205	3.9	4.7	13:45	189	
3205	3.4	8.3	17:30	200	
3205	4.4	11.3	17:30	214	
3205	5.3	11.0	15:00	229	
3205	8.1	2.7	09:30	238	Precipitation night before
3205	10.1	4.2	17:00	252	
3205	12.0	8.6	16:45	275	

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