Overview:

The McMurdo Dry Valleys (MDVs) are a cold desert region of Antarctica supporting terrestrial and aquatic ecosystems that host microbial food webs, with few species of metazoans and no vascular plants or vertebrates. Biota have adapted to the cold, dark, and arid conditions that prevail for all but a brief period in the austral summer. In the summer, soils warm and glacial meltwater flows through streams into the open moats of permanently ice-covered, closed-basin lakes. Because of this energy, water, and ecological connectivity, most biological activity across the landscape occurs in summer. Through the winter, or polar night (6 months of darkness), glaciers, streams, and soil biota are inactive until sufficient light, heat, and liquid water return, while lake communities remain active all year. The McMurdo Dry Valleys LTER (MCM LTER) has studied this ecosystem since 1993. The last two cycles of the MCM LTER have documented dynamic landscape and ecosystem responses to recent warming and enhanced connectivity. Over the past 30 years, the MDVs have been disturbed by cooling, heatwaves, floods, rising lake levels, and permafrost and lake ice thaw. Considering the clear ecological responses to this variation in physical drivers, and climate models predicting further warming and more precipitation, the MDV ecosystem sits at a threshold between the familiar extreme cold and dry conditions and an uncertain future. In MCM6, we will examine how changes in the temporal variability of ecological connectivity interact with the existing landscape legacies that have defined habitat suitability and biogeochemical cycling for millennia. We hypothesize that:

The structure and functioning of the MDV ecosystem is dependent upon legacies and the contemporary frequency, duration, and magnitude of ecological connectivity.

This hypothesis will be tested with new and continuing monitoring, experiments, and analyses of our long-term datasets to examine: **1)** the stability of MCM ecosystems as reflected by sentinel taxa, **2)** the relationship between ecological legacies and ecosystem resilience, **3)** the importance of material carryover during periods of low connectivity to maintaining biological activity and community stability, and **4)** how changes in disturbance dynamics disrupt ecological cycles through the polar night.

Intellectual Merit:

In MCM6 we will test ecological connectivity and stability theory in a system subject to strong physical drivers (geological legacies, extreme seasonality, and contemporary climate change) and driven by microbial organisms. Since microorganisms regulate most of the world's critical biogeochemical functions, these insights will be relevant far beyond polar ecosystems and will inform understanding and expectations of how natural and managed ecosystems respond to ongoing anthropogenic global change. MCM6 builds on previous foundational research, both in Antarctica and within the LTER network, to consider the temporal aspects of connectivity and how it relates to ecosystem stability.

Broader Impacts:

We have developed six Antarctic Core Ideas which encompass themes from data literacy to polar food webs – these big ideas form a consistent thread across our education and outreach activities. Building on past success, we will establish collaborations with teachers and artists who, embedded within our science teams, will work to develop educational modules with science content informed by direct experience and artistic expression. Our undergraduate mentoring efforts are evolving to incorporate computational methods through a new data-intensive scientific training program for MCM REU students. We will also establish an Antarctic Research Experience for Community College Students at CU Boulder, to provide an immersive educational and research experience to students from diverse backgrounds in community colleges. MCM LTER will continue its mission of training and mentoring students, postdocs, and early career scientists as the next generation of leaders in polar ecosystem science and stewardship. We will expand historically underrepresented participation at each level of the project. To aid in these efforts, we have established *Education & Outreach* and *Diversity, Equity, and Inclusion* committees to lead, coordinate, support, and integrate these activities through all aspects of MCM6.

This proposal requires fieldwork in the Antarctic.

LTER: MCM6 - The roles of legacy and ecological connectivity in a polar desert ecosystem

The McMurdo Dry Valleys (MDVs; 78°S, 162°E) represent the largest (4500 km^2) ice-free area on the Antarctic continent (Fig. 1; Levy 2013). The MDV landscape is a mosaic of glaciers, exposed soils and rock, ephemeral stream channels, and closed-basin ice-covered lakes. Given its polar location, the MDVs have constant sunlight through the austral summer (Oct-Feb) and perpetual darkness through the austral winter (Jun-Aug). The mean annual air temperature of the MDVs is -19°C, with extreme cold in the winter (< – 40°C) and temperatures just above freezing in the summer. Soils host a limited diversity of microbes, protists, and microinvertebrates, and streams create extensive habitat for benthic cyanobacterial mats and diatom communities (Alger et al. 1997, Kohler et al. 2015b). Hyporheic zones surrounding streams are critically important to both high weathering rates (Gooseff et al. 2002, Nezat et al. 2001) and biogeochemical cycling (Gooseff et al. 2004, McKnight et al. 2004, **Singley et al. 2021**), and mobilizing nutrients that support down-stream biota (**Kohler et al. 2018**). Lakes are strongly stratified under 3-5 m of permanent ice cover, some with hypersaline waters at depth, and host communities of photosynthetic and mixotrophic phytoplankton (Priscu et al. 1999, Dolhi et al. 2015), while predatory heterotrophic nanoflagellates and haptorid ciliates represent the apex predators of the lake food webs (**Li and Morgan-Kiss 2019**). The edges of the lakes melt out each summer creating "moats" with extensive cyanobacterial mats and directly interact with the atmosphere, stream inflows, and the deeper water column under the perennial ice. Even MDV glaciers host microcosms of life in cryoconite holes collecting aeolian deposited sediments and microorganisms (Christner et al. 2003, Porazinska et al. 2004).

In the MDVs geological legacies imprint the physical and chemical structure of the landscape over thousands to millions of years (Fountain et al. 1999, Hall 2009). These material legacies (*sensu* Johnstone et al. 2016) influence the contemporary structure and functioning of terrestrial and aquatic ecosystems in distinct ways across the linked landscapes of the MDVs: in soils the distributions of salts, nutrients, and organic matter are influenced by the glacial and lacustrine history of the region; stream dynamics are driven by geomorphic legacies and the geographic position of glaciers and their mass balances; and lake biogeochemistry is influenced by both their landscape position with respect to glacial till mineralogy and

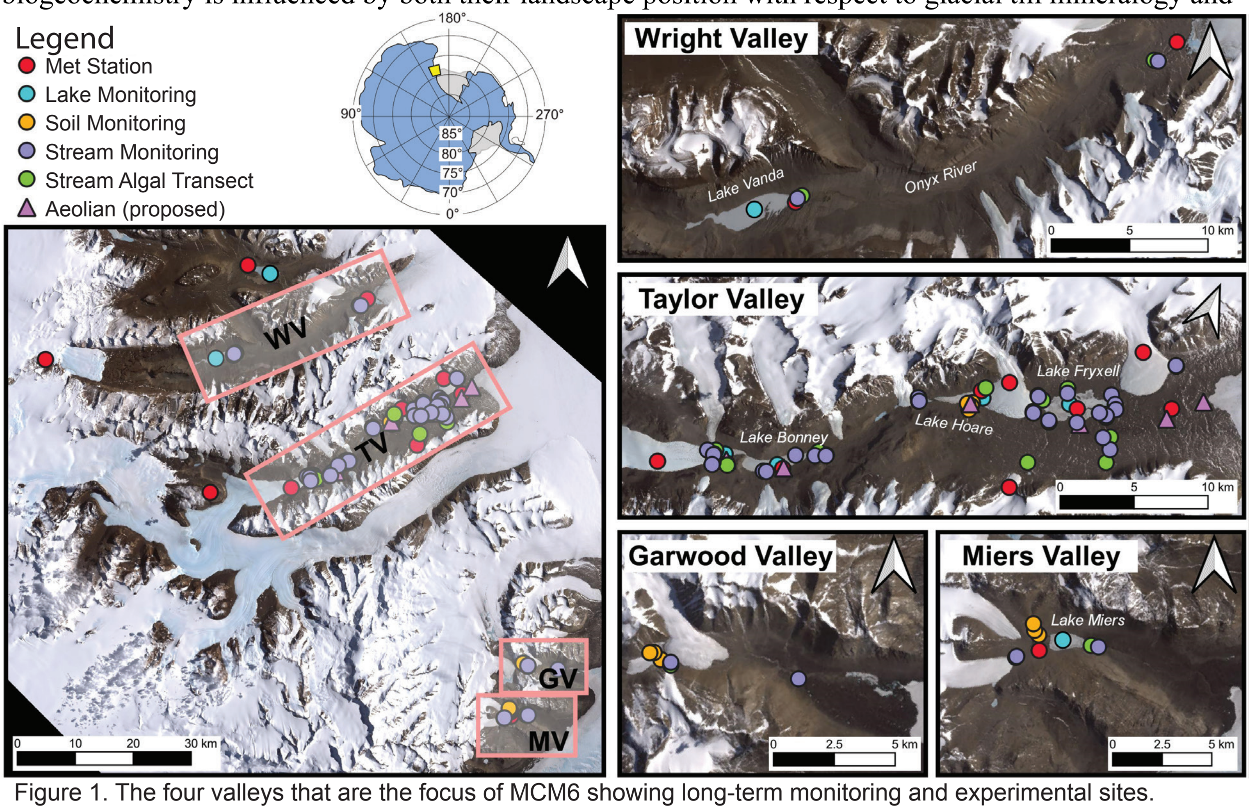

Figure 1. The four valleys that are the focus of MCM6 showing long-term monitoring and experimental sites.

nutrient content and their bathymetry that together determine the highly stable physical, chemical, and biological stratification (Priscu 1995, Lyons et al. 2000, Barrett et al. 2007). These legacies are modulated by contemporary processes which redistribute soil, sediment, water, and organisms across the landscape.

Ecological connectivity theory describes how spatial structure and temporal dynamics of ecosystems are regulated by movement of material (i.e., water, dust, sediment) and biota (Peters et al. 2018, 2020, Iwaniec et al. 2021). Movements of organisms and matter are facilitated by physical and biological processes that interact over multiple spatial and temporal scales (Peters et al. 2018). The concept of connectivity helps elucidate interactions among processes occurring over different scales, such as when processes at one scale are overridden by processes at another, as occurs in fire and disease transmission (Iwaniec et al. 2021), permafrost thaw/degradation affecting soils and sediments (Gooseff et al. 2016), and floods (Peters et al. 2018). Ecological connectivity can enhance ecological resilience, especially in terms of diversity where inter-patch dynamics can maintain regional diversity even when local diversity changes (**Sokol et al. 2020**). However, under increased global change, connectivity may lead to alternative stable states where changes in physical drivers are amplified across landscapes (O'Donnell et al. 2011, Lafreniere and Lamoureux 2019).

The MDV polar desert ecosystem is connected physically by wind and water across different spatial and temporal scales (Fig. 2). Strong foehn winds (most common in the winter) redistribute mineral and biological material around the MDVs (Šabacká et al. 2012, **Diaz et al. 2018**, Schulte et al. 2022). Windstorms may last for hours or days with long hiatuses between events, resulting in strong temporal variations in landscape connectivity. During summer, the ecosystem becomes hydrologically connected for 6-12 weeks as glaciers melt at their surface and feed stream channel flows, most of which in turn feed closed-basin lakes (some flow out to McMurdo Sound). Thus, summer is the season of highest biological activity and connectivity across the landscape. In fall, temperatures cool, daylight fades, glacial melt ceases, and the ecosystem becomes hydrologically disconnected. Between flow seasons, glacier, stream, and soil biota desiccate or enter a suspended state until sufficient light, heat and liquid water return the next summer. However, under the cover of ice, liquid water persists, and lake biota continue to be active in winter (Takacs and Priscu 1998) with some algal populations continuing to grow (**Patriarche et al. 2021**). *The temporal dynamics of these physical and biological connections and their links to ecosystem and community resilience are the focus of MCM6.*

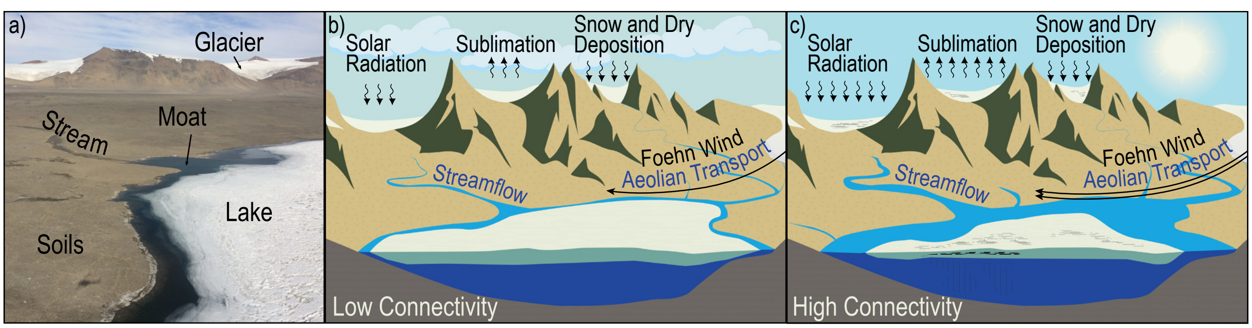

Figure 2. a) Landscape of the McMurdo Dry Valleys, and our conceptual models of the physical differences across the landscape in states of b) low connectivity, and c) high connectivity.

1. RESULTS FROM PRIOR SUPPORT

The MCM5 PI team included: Adams (evolutionary ecologist), Barrett (soil ecologist/biogeochemist), Doran (hydrogeologist/paleolimnologist), Gooseff (PI, hydrologist/modeler), Howkins (environmental historian), McKnight (stream ecologist/hydrologist), Morgan-Kiss (microbial ecologist), Priscu (limnologist/ biogeochemist), and Takacs-Vesbach (microbial ecologist/limnologist). We involved 44 graduate students, 45 undergraduate students (including 15 REUs), 6 postdoctoral fellows, and nearly 8,000 K-12 students, teachers, and families combined. We also involved more than 40 collaborators in MCM5.

1.1 Research Products and Data Availability.

During MCM5, we produced 115 papers in refereed journals (see *Table 1 for the 10 most representative*), 5 book chapters, 2 conference proceedings, 1 report, and 24 theses/dissertations (see *Supplementary Documents*). More than 300 MCM datasets spanning the five LTER core research areas are available through the online MCM Data Catalog as well as the Environmental Data Initiative (EDI) repository, which is a member node of DataONE (see *Dataset Inventory*). MCM data are also discoverable via metadata records in the Antarctic Master Directory (AMD) and the US Antarctic Program Data Center (USAP-DC), with data links pointing to the MCM Data Catalog and to the master list of MCM datasets in EDI (see *Data Management Plan* for details).

Table 1. **Ten Most Representative Papers from MCM5 (2017-2022).** Papers were chosen for impact, author diversity, long-term data use, LTER core areas, and MCM6 research goals. Graduate students and postdocs are underlined, and significance is noted in green. These citations are identified in **bold font** throughout this proposal.

Aanderud et al. 2018. Stoichiometric shifts in soil C:N:P promote bacterial taxa dominance, maintain biodiversity, and deconstruct community assemblages. *Front. Microbiol.* 9:1401. CNP additions to nutrient-limited soils result in changes in species dominance and deconstructs species interconnectedness to the maintenance of biodiversity and functioning.	Li and Morgan-Kiss. 2019. Influence of environmental drivers and potential interactions on the distribution of microbial communities from three permanently stratified Antarctic lakes. *Front. Microbiol.* 10:1067. MDV lake protists are spatially segregated within the stratified water column and have lake-specific potential interactions with bacterioplankton.
Andriuzzi et al. 2018a. Observed trends of soil fauna in the Antarctic Dry Valleys: Early signs of shifts predicted under climate change. *Ecology* 99:312–321. Climate change is driving changes in the diversity, distribution and abundance of MDV soil fauna.	Obryk et al. 2020. Climate from the McMurdo Dry Valleys, Antarctica, 1986–2017: Surface air temperature trends and redefined summer season. *J. Geophys. Res. Atmos.* 125:e2019JD032180. Redefined the seasons in the MDVs based on solar radiation, wind and air temperature data. Updated all MDV climate averages 17 years after previous publication.
Diaz et al. 2018. Aeolian material transport and its role in landscape connectivity in the McMurdo Dry Valleys, Antarctica. *J. Geophys. Res. Earth Surf.* 123:3323–3337. Water-soluble nutrient ratios in aeolian material vary seasonally and spatially in Taylor Valley, suggesting that winds source limiting nutrients.	Patriarche et al. 2021. Year-round and long-term phytoplankton dynamics in Lake Bonney, a permanently ice-covered Antarctic lake. *J. Geophys. Res. Biogeosci.* 126:e2020JG005925. Phytoplankton communities accumulate chlorophyll during the winter, owing to a bloom in mixotrophic haptophytes.
Gooseff et al. 2017a. Decadal ecosystem response to an anomalous melt season in a polar desert in Antarctica. *Nat. Ecol. Evol.* 1:1334–1338. Ecosystem components rebound from disturbance on different timescales.	Singley et al. 2021. The role of hyporheic connectivity in determining nitrogen availability: Insights from an intermittent Antarctic stream. *J. Geophys. Res. Biogeosci.* 126:e2021JG006309. Remineralized autochthonous N accumulates in hyporheic zones subsidizing stream N availability, thus highlighting the importance of hyporheic connectivity.
Kohler et al. 2018. Catch and release: Hyporheic retention and mineralization of N-fixing Nostoc sustains downstream microbial mat biomass in two polar desert streams. *L&O Letters* 3:357–364. N in microbial mats is recycled and exported, maintaining downstream mat communities; organic matter cycling supports primary production in N-limited streams.	Sokol et al. 2020. Evaluating alternative metacommunity hypotheses for diatoms in the McMurdo Dry Valleys using simulations and remote sensing data. *Front. Ecol. Evol.* 8:521668. Data catalog records of diatom community composition and metacommunity simulations indicate that dispersal in addition to species sorting is necessary to explain regional diversity patterns.

1.2 Results of Broader Impacts: Education and Outreach Activities.

Outreach and education work in MCM5 focused on coordinating activities across PI institutions, with CU Science Discovery leading science communication training and support for graduate students and researchers. We incorporated MCM data into teacher professional development workshops for elementary schools, reaching 1482 students and 296 teachers in primarily rural schools. PI McKnight brought our schoolyard book, *The Lost Seal* (McKnight 2006), to a variety of large science and engineering festivals, engaging with thousands of children and adults. During the 2018-19 field season, Kathryn Penzkover (CU Science Discovery) engaged the science support community on the ice with MCM research, including a popular board game (Antarctic-catan) and a Cafe Scientifique lecture series. Education and outreach coordinator Dr. Alex Rose and PI Morgan-Kiss collaborated with the Palmer LTER (PAL) on an NSF Advancing Informal STEM Learning (AISL) grant to bring the Polar Literacy principles into informal learning settings. Although the implementation of this project was hampered by the Covid-19 pandemic, the team developed a teaching module using MCM lake sequence data and introduced >200 students to MCM science. We hosted a PolarTREC teacher, Kevin Dickerson, and maintain annual classroom visits to kick-off their LTER research projects and visits to BYU to present their results. PI Morgan-Kiss worked with an artist, Xavier Cortada, on an integrated science-art communication project with an undergraduate class. We worked with the CU Museum of Natural History to host an Antarctic Family

Day for ~250 visitors of all ages. We developed an exhibit about MCM research called *Antarctica: More than Meets the Eye*, which is the museum's first entirely bilingual (English & Spanish) exhibit. PI Doran started a virtual MCM LTER Science Café. PIs Takacs-Vesbach and Diaz organized two special sessions at SACNAS about MCM and interdisciplinary science to encourage diversity in polar research. We have also created a new education and outreach website that serves as a platform to share resources, information, and outreach news, focused on teachers and students.

1.3 Results of Supplemental Support.
During MCM5, we received three supplements. The first supplement (2018) was used to replace an ion chromatograph at McMurdo Station to analyze major ions from stream and lake samples during deployment, reducing sample shipping costs and ensuring time sensitive samples are properly quality assured and controlled. The second supplement (2019) supported the replacement of a US Antarctic Program (USAP) CTD instrument (>20 years old) used for lake sampling and profiling measurements. The third supplement (2021) supported research assistantships for four graduate students who deployed to Antarctica in the long 2021-22 field season (long quarantines and few flights to/from Antarctica) and would have otherwise been able to be supported as TAs at their home institutions.

1.4 Ecosystem Responses in the McMurdo Dry Valleys.
Ecological stability in the face of global change is essential to maintaining the regulatory, provisioning, and aesthetic ecosystem services of the biosphere (Millennium Ecosystem Assessment (MEA) 2005). Stability, defined as the resistance of an ecosystem to change or disturbance and the recovery from that change, i.e., resilience (Table 2), is an enduring paradigm in ecology and increasingly in the social and economic sciences (Holling 1973, Pimm 1986, Tilman and Downing 1994, Carpenter et al. 2001, MEA 2005, Cumming and Peterson 2017). Stability is not an obvious concept to test in the MDVs because much of the ecosystem structure appears highly stable and insensitive to interannual variation. For example: microbial mats and soil biota can remain in dormant freeze-dried, anhydrobiotic, or sporulated states for decades (Treonis et al. 2000, Adams et al. 2006, McKnight et al. 2007, Schwartz et al. 2014) and are exceptionally slow growing when active (Johnston and Vestal 1991, Overhoff et al. 1993, Sun and Friedmann 1999); lake stratification is stable for thousands of years (Lyons et al. 2000); and the land surface itself is a legacy of geomorphic processes occurring over thousands to millions of years (Fountain et al. 1999, Hall 2009). Yet, this historically stable ecosystem is vulnerable to major ecological shifts as regional climate changes toward warmer and wetter conditions, as is predicted for the future (Walsh 2009, Rintoul et al. 2018, Bracegirdle et al. 2020, IPCC 2022). Warming in the region will lead to significant environmental changes including increased frequency, magnitude, and duration of extreme meteorological events (e.g., rain, which was observed for the first time in summer 2018-19). Coastal Antarctic precipitation is expected to increase 8-31% depending on the emissions scenario (Bracegirdle et al. 2020) with more rain (Vignon et al. 2021). Such changes in regional climate would profoundly alter the governing physical drivers of the MDV ecosystem because of the inherent sensitivity of polar ecosystems to even modest shifts in climate, i.e., polar amplification (Goosse et al. 2018).

Recognizing the importance of climate events such as the 2001-02 flood year (**Gooseff et al. 2017a,** Doran et al. 2010) and learning from our manipulative experiments over the last two LTER cycles, it is clear that disturbances (Table 2) have distinct and important effects across the MDV ecosystem. Further, the temporal dynamics of the resident biota and the physical environments in which they reside influence their resistance and resilience to these changes. In this iteration of the MCM LTER program (MCM6), we will examine the responses of this polar desert ecosystem to disturbances manifest as anomalous weather events and climate change (e.g., high melt/thaw years, wetting/flooding, permafrost degradation, wind-sediment redistribution, and related manipulative experiments). Below we summarize the primary results of our research, modeling, and synthesis from MCM5 and how our greater understanding of connectivity in the MDVs has led to the development of our improved conceptual framework and models for MCM6.

Table 2. Key terms and definitions

Sentinel or Indicator Taxa: a group of organisms that play key role(s) in carbon/nutrient cycles and represent biological indicators of changes in the environment (Thorpe et al. 2016). *E.g., soil nematode taxa are climate-sensitive drivers of heterotrophic carbon flux (Barrett et al. 2008); chlorophyte and cryptophyte taxa exhibit spatial and temporal trends in MDV lakes (Dolhi et al. 2015; Patriarche et al. 2021).*

Carryover effect: Events and/or resources from one season that have impacts on a subsequent season. A measurable change in the current status of an ecosystem or activity/abundance of a group of organisms can be explained by a previous change in the system (O'Connor et al. 2014). *E.g., stimulation in summer phytoplankton productivity during a flood year can provide increased biomass for winter heterotrophic activity (Patriarche et al. 2021).*

Resistance (from MCM5): The ability of an ecosystem to withstand a disturbance and associated stress (sensu Pimm 1984). *E.g., soil nematode populations are relatively unchanged by mild trampling disturbance (Ayres et al. 2008).*

Resilience (from MCM5): The capacity of an ecosystem to recover from disturbance and stress (Holling and Gunderson 2002); the magnitude of a disturbance (*e.g.,* degrees of temperature, stream flow, or physical disturbance) tolerated by an ecosystem before significant change in structure and/or functioning is detected (Holling 1973). *E.g., rapid recovery of algal mat and diatom populations following removal (Kohler et al. 2015a).*

Ecological stability is a combination of resistance and resilience (Holling 1973) where **resistance** is the ability of an ecosystem to withstand a disturbance and associated stress (sensu Pimm 1984), and **resilience** is the capacity of an ecosystem to recover from disturbance and stress (Holling and Gunderson 2002). *E.g., soil nematode populations are resistant to mild trampling disturbance (Ayres et al. 2008), and resilience is illustrated by the rapid recovery of algal mat and diatom populations following scour (Kohler et al. 2015a).* We will calculate resistance and resilience indices originally developed by Orwin and Wardle (2004). →

Resistance

$$\text{RS}\ (t_0) = 1 - \frac{2|D_0|}{(C_0 + |D_0|)}$$

Resilience

$$\text{RL at } t_x = \frac{2|D_0|}{(|D_0| + |D_x|)} - 1$$

where D_0 = diff. from control (C_0) at end of disturbance (t_0). D_x = diff. from control at time point (t_x)

1.4.1 Meteorology - Meteorological data are used to define the abiotic variability of the MDVs and are a core part of the MCM Data Catalog. Our 30-year record is used to define the contemporary climate of the MDVs. The mean annual temperature ranges from -15°C to -30°C and mean annual solar radiation varies between 72 and 122 W m^{-2} (**Obryk et al. 2020**). Doran et al. (2002a) showed that air temperature decreased by 0.7°C per decade between 1986-2000, with **Obryk et al. (2020)** showing that this cooling trend continued to 2006 and has since been highly variable with no trend. Winds are important in transporting mineral and biological material across the ecosystem (Šabacká et al. 2012, **Diaz et al. 2018**, Schulte et al. 2022). Doran et al. (2008) concluded that the warmest summers are associated with increased frequency of foehn winds. Fountain et al. (2010) defined the spatial and temporal variation of precipitation across the valleys and identified a strong gradient with less precipitation further from the coast. Similarly, Acosta et al. (2020) show that cloudiness generally decreases away from the coast. Myers et al. (*In revision*) found that snowfall averaged 11 mm water equivalent from 1995 to 2017 in Taylor Valley (excluding winter) with a range of 1 to 58 mm. An inflection occurred in 2007 from more to less snowfall which coincides with the end of the cooling trend in 2006. Even small changes in temperature and solar radiation seem to affect the MDV ecosystem because local climate is poised on a threshold between melting and freezing of water (Fountain et al. 2014).

1.4.2 Glaciers, Active Layer, & Permafrost - In MCM5, we evaluated land surface albedo across Taylor Valley and found that spatial patterns of albedo are a function of landscape morphology, persisting at high levels where snow is trapped in depressions and lees (Bergstrom et al. 2020a). Analyses of long-term records of glacial mass balance and streamflow and two digital elevation models of the MDVs revealed that annual streamflow is generally greater than the glacier mass loss observed, and that this imbalance grew after the flood year of 2002 (Bergstrom et al. 2021). This difference is largely due to the roughening of the glacier surfaces between 2001 to 2014 which enhanced melt generation from increased vertical ice surfaces (and related increased surface area), which are unaccounted for in traditional glacial mass balance measurements. During MCM5 we also discovered that supraglacial streams are biologically active, retaining nitrate and phosphate and exhibiting clear metabolic signatures similar to MDV streams (Bergstrom et al. 2020b). We also established new Active Layer Monitoring Stations (ALMSs; 2 adjacent to streams, 2 adjacent to water tracks, and 1 in dry soil) and Active Layer and Moat Monitoring Stations (ALMMS) to support our new research focus on the moats of Lakes Fryxell and Bonney. These stations measure soil temperature, soil moisture, and specific conductance (SpC) through the active layer (surface down to the frost table) at several locations from the water's edge to dry soils. These data have supported new insights into the thermal-moisture dynamics of soils that may control habitat conditions and faunal responses to seasonal and annual freezing cycles (Wlostowski et al. 2018a). Finally, we also established a new permafrost thermistor array to a depth of 30 m (in an old borehole) to initiate the long-term monitoring of ground temperatures. Glaciers, active layer, and permafrost underlie the MDVs ecosystem and are vulnerable to climate warming. They are also active components of the ecosystem.

1.4.3 Soils - Soil biotic communities in the MDVs are characterized by low biomass and include cyanobacteria, archaea, bacteria, fungi, protozoans, and a limited diversity of metazoan invertebrates (Adams et al. 2006, Cary et al. 2010, Lee et al. 2019, Thompson et al. 2021). While earlier models of soil ecosystems mainly considered legacy distributions of lacustrine organic matter as the source of energy fueling MDV soil food webs (Burkins et al. 2000, 2001), more recent work has demonstrated the importance of contemporary C fixation to soil communities (Geyer et al. 2017, Shaw et al. 2018, Geyer and Barrett 2019, Power et al. 2020). Metazoan species richness, composition, and productivity are influenced by physicochemical characteristics including water content, organic matter, and soluble salt content (Poage et al. 2008, Caruso et al. 2019). Notably, the most common nematode taxa in Taylor Valley (*Scottnema lindsayae*, *Plectus* spp, and *Eudorylaimus* spp) exhibit distinct habitat preferences (Fig. 3), with *Plectus* prevalent in saturated environments, *S. lindsayae* occupying the vast expanses of dry soils, and *Eudorylaimus* found in saturated and intermittently wet environments (Treonis et al. 1999, **Andriuzzi et al. 2018a**, Caruso et al. 2019). These metazoan populations have exhibited significant variation in abundance associated with climate variation over the past 30 years (Doran et al. 2002b, Barrett et al. 2008b, **Gooseff et al. 2017a**), and exhibit community-level changes consistent with early models of habitat suitability (Freckman and Virginia 1997, **Andriuzzi et al. 2018a**). These changes have illustrated how contemporary variability in water availability can modulate the influence of legacies on the diversity, distribution, and activity of soil organisms (**Andriuzzi et al. 2018a**, 2018b, Geyer and Barrett 2019).

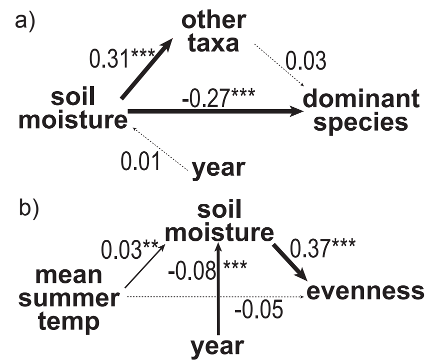

Figure 3. Structural equation models for (a) *S. lindsayae* and sub-ordinate soil invertebrate species abundance at elevational transect studies in the three valleys (2003–2017, n = 324), and (b) Pielou's evenness in Taylor Valley (1994–2015, n = 267). Numbers on arrows are standardized coefficients with significance (**P < 0.001, ***P < 0.0001) (**Andriuzzi et al. 2018a**).

1.4.4 Streams - Streams are the primary conduit connecting glaciers and soils to the lakes. After the benchmark flood year of 2002, decadal trajectories of flow increased immediately, while distinct microbial mat communities exhibited different lags in growth following higher water availability (**Gooseff et al. 2017a**). Higher flows through the extensive stream hyporheic zones are associated with higher solute concentrations from greater weathering (Gooseff et al. 2017b, Wlostowski et al. 2016, 2018b), which changes the osmotic conditions that in-stream organisms must cope with to maintain cellular integrity. Furthermore, biota within these hyporheic zones assimilate organic nitrogen (N) that is transported from N-fixing benthic mats upstream (**Kohler et al. 2018**), and subsequently mineralize this N, making hyporheic zones a source of nitrate relative to the stream channel (**Singley et al. 2021**). The influence of hyporheic zone solute flushing through fluctuations in stream flow appears to be stronger on daily time scales (flow is high during brighter hours, low during darker hours), while low-flow years trend towards higher solute concentrations overall due to evaporative processes (Singley et al. 2017). Primary producers in streams are limited by N availability, and biological release from N limitation at local scales changes algal communities by increasing the abundance of cyanobacteria and cosmopolitan diatom species (Kohler et al. 2016). In contrast, variation in the diversity of bacteria and Eukarya among streams seems to be related more to flow variability than stream chemistry. However, further consideration of top-down controls on stream production is warranted, given the consistent presence of rotifers, nematodes, and tardigrades in benthic mats (Van Horn et al. 2016), and the higher abundance of benthic mat associated eukaryal grazers in a dry than a wet year (Andriuzzi et al. 2018b). Wetter habitats in both the stream channel and hyporheic edge, even if saltier, may be more hospitable for organisms in these higher trophic levels (Wlostowski et al. 2018a, 2019).

1.4.5 Lakes - MDV lakes integrate environmental change within each basin and are the only landscape unit that supports year-round liquid water and metabolic activity. The limnology team has measured selected physical, chemical, and biological parameters in four lake basins within the Taylor Valley (Fryxell [FRX], Hoare [HOR], East and West Lobe Bonney [ELB and WLB, respectively]; Fig. 1) approximately monthly from November to January since 1994. Long-term data on phytoplankton dynamics reveal that summer rates of primary productivity (PPR), phytoplankton particulate organic carbon (POC), and phytoplankton C turnover time respond to changes in temperature-driven stream flow (**Gooseff et al. 2017a**). Year round high-frequency measurements of photosynthetically active radiation (PAR) are used to model PPR at 10 m below the water/ice surface (**Gooseff et al. 2017a**).

The decade before the flood year in 2002 was a period of cooling and ice thinning, and a decreasing trend in phytoplankton production (Doran et al. 2002b). After 2002, depth-integrated chlorophyll-a (chl-a) immediately increased, caused by stimulation of green algae. In contrast with these long-term trends, a mixed population dominated by a mixotrophic haptophyte (Li et al. 2016, **Li and Morgan-Kiss 2019**) is the major contributor to mid-winter chl-a accumulation during the polar night (Fig. 4; **Patriarche et al. 2021**). These results have led us to develop a conceptual model of the lake ecosystem where periods of relatively stable climate are punctuated by decadal warming events. Introduction of 16S and 18S rRNA gene diversity as a new monitoring activity has revealed communities with distinct spatial trends among the lakes and by depth (Kwon et al. 2017, **Li and Morgan-Kiss 2019**). Sampling was limited to summer until we deployed the automated lake profiling samplers (ALPS), which revealed marked seasonal differences among plankton (§3.1.7; **Patriarche et al. 2021**). Manipulation experiments have identified specific bacterial and phytoplankton taxa that are responsive to climate-related environmental disturbances (§3.1.8; **Li and Morgan-Kiss 2019**, Sherwell et al. *In review*).

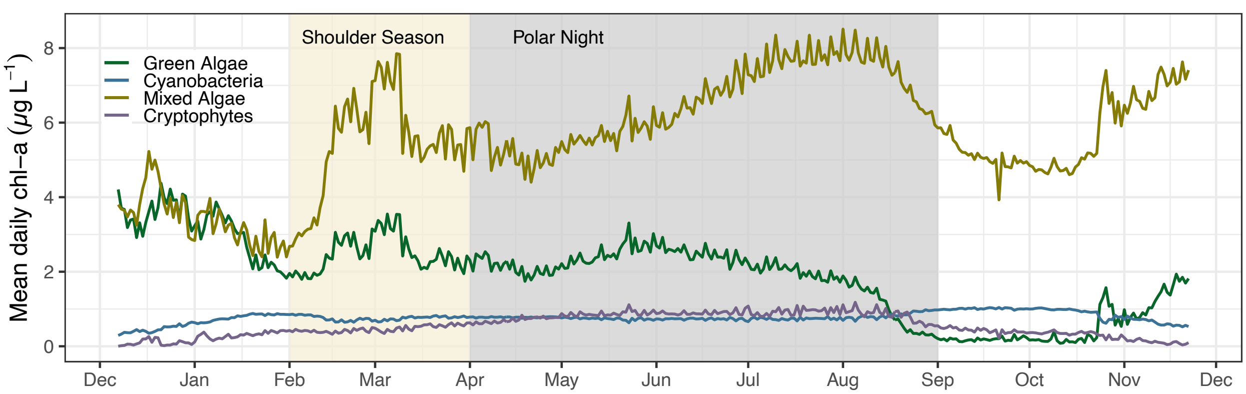

Figure 4. WLB ALPS fluoroprobe mean chl-a concentration of four algal groups between 16-19 m in 2014.

1.4.6 Integrative Responses Across Landscape Units and Ecological Modeling - Higher stream flows in the late 2000's maintained solute concentrations (i.e., chemostasis, Wlostowski et al. 2018b) and increased solute flux to downstream lakes and marine habitats, which has implications for relief of micronutrient (e.g., Fe and S) limitation to biological processes and possible acceleration of primary production in downstream habitats (Olund et al. 2018). Lake level rise and increased hydrological connectivity across soils, moats, and stratified water columns may increase homogenization (§3.2.3). The importance of regional connectivity of wind-transported chemicals, which may further promote biological activity, is also supported by data showing widespread aeolian movement of major and trace nutrients through the MDVs (**Diaz et al. 2018,** Diaz et al. 2020).

Modeling suggests that regional dispersal is necessary to support the standing levels of diversity in Antarctic diatom communities, and that neither neutral nor niche metacommunity mechanisms are sufficient to explain observed biodiversity patterns (Sakaeva et al. 2016**,** Sokol et al. 2017, **Sokol et al. 2020**). Further investigation confirmed the presence of a diversity of diatom taxa, including many viable cells (with cytoplasm and/or RNA) in aeolian material collected from three basins across the MDVs (Fig. 5) (Schulte et al. 2022). Furthermore, the aeolian community composition was intermediate among all other diatom habitats sampled (cryoconite, lake, stream; Schulte et al. 2022). Alongside evidence for strong co-occurrence of bacterial and eukaryal taxa among cryoconite holes region-wide (Sommers et al. 2018), it is clear that wind is a homogenizing factor that supports long-range dispersal of biota among habitats across the region.

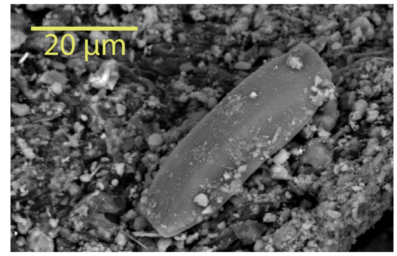

Figure 5. Scanning electron microscope image of aeolian diatom frustule (*Hantzschia amphioxys*).

1.4.7 Environmental History - In MCM5 we used an historical framework to examine the combined influences of human activity, scientific research, and environmental change over the past century (Chignell et al. 2021, Kohler et al. 2021). Archival research on the history of human activity in the MDVs was performed in the US, New Zealand, Japan, Australia, and the United Kingdom, and by oral history interviews conducted with over 25 scientists who have worked in the region since the 1950s. This work, which is available as a digital archive through the MCM Data Catalog (Howkins et al. 2020), is being developed into an academic monograph on the environmental history of the MDVs, and has already had an impact by informing Antarctic environmental management policies (Priscu and Howkins 2016). We also sought to understand whether disturbance via history of human activities increases connectivity and accelerates shifts towards homogeneity in soil ecosystems. We found nematode abundance was especially sensitive to traces of human activity in old field camps, relative to soil bacterial communities.

1.4.8 Intersite Comparisons - In MCM5, we collaborated with several other LTER sites to contribute to syntheses of ecological connectivity across the LTER Network (Iwaniec et al. 2021) and climate change in dryland ecosystems (Hudson et al., *In press*). We have been involved with several LTER Network synthesis working groups to investigate: ecological metagenome-derived reference genomes and traits (PI Takacs-Vesbach, Investigator Stanish); controls of riverine exports of silicon across biomes (McKnight, Investigator Heindel); and how metacommunity dynamics influence community responses to disturbance across the Network (Investigator Sokol). NTL PIs and investigators (including McKnight and Dugan) participated in a 2019 AGU Chapman conference on winter limnology, with contributions to an AGU *JGR-Biogeosciences* special issue (**Patriarche et al. 2021**, Cavaliere et al. 2021). PI Barrett and Investigator Heindel led an LTER connectivity session at the 2018 Ecological Society of America Meeting. Information Manager Brown collaborates frequently with others across the LTER Network. For example, she contributed to the development of a best practice guide on archiving non-tabular data types (Gries et al. 2021). Morgan-Kiss and Rose collaborated with Palmer LTER to bring Polar Literacy principles into schools.

1.5 Integrating Input from the MCM5 Site Review

Following suggestions from site review in Jan 2020, we have modified our leadership structure to include PIs and non-PIs in twice-monthly leadership group meetings, and established a new Diversity, Equity, and Inclusion (DEI) committee. In MCM6, we will add four new committees: Education & Outreach (E&O), External Image, Information Management, and Sustainability (see *Project Management Plan*). We modified our research plan to maximize resources for the most cutting-edge areas of research that can incorporate new technologies and scientific advancements. For example, we retired the P3 (Pulse Press Permafrost) experiment and scaled down some limnology and stream monitoring activities to develop new experiments focused on differing scales of (dis)connectivity due to landscape-wide change, such as Decreased Irradiance on Mats (DIM, §3.3.4), Seasonal Soil Manipulation (SSM, §3.3.1), Permafrost Subsidence Experiment (PSE, §3.3.2), Narrows monitoring (§3.1.7), and Re-relict Channel experiment (§3.2.2). We are also incorporating remote sensing into our monitoring and experiments to characterize the spatial and temporal variability of these ecosystems across the landscape.

Following recommendations for our Information Management System (IMS), we completed major upgrades of technical infrastructure, which included the migration of our databases from Oracle to

PostgreSQL, and new website sections on how to access and submit MCM data. The migration of our bibliography to Zotero is nearly complete, which will assist in tracking data citations and data reuse.

To reflect our efforts to integrate our education and outreach efforts across MCM, we developed a new outreach website and are implementing a teaching module emphasizing 6 core ideas about Antarctica (§5.1.1). We were encouraged to increase our efforts to recruit more team members from historically underrepresented groups. We have responded by diversifying our leadership team and will recruit new polar researchers in an evidence-based REU training program in MCM6 (§5.2.1).

1.6 Covid-19 Impacts on the MCM LTER.

The pandemic halted operations just two months after our MCM5 site review. Immediate impacts to our project were felt through long delays in accessing our laboratories to analyze samples from the 2019-20 field season. In 2020-21, our team did not deploy to the field, and in 2021-22, only 10 personnel deployed after 3+ weeks of quarantine, i.e., both personnel and time in the field was much reduced (compared to a normal deployment of 31 people). Thus, we have gaps in most of our long-term datasets, especially those related to biological processes that require direct field sampling or measurements.

2. PROPOSED RESEARCH

The research proposed in MCM6 will be addressed by a team consisting of 5 new early to mid-career PIs (Diaz, Dugan, Mackey, Salvatore, Zeglin), and 6 senior PIs carrying over from MCM5 (Adams, Barrett, Doran, Gooseff, Morgan-Kiss, Takacs-Vesbach). Five of our PIs identify as female, 6 as male, and 2 are from historically underrepresented communities. Our research is also supported by two foreign scientists with MDV experience: Dr. Tyler Kohler (starting at Charles University in Prague July 2022), an expert on Antarctic diatoms, and Dr. Ian Hawes (Waikato University, New Zealand), an experienced researcher in Antarctic lake benthic communities and diving operations (see *Letters of Collaboration*).

2.1. Introduction.

In MCM4 and MCM5 we progressed from a landscape legacy perspective to focus on ecological connectivity as a driver of ecosystem structure and functioning (Fig. 6). The working hypotheses of MCM4 stated expectations that positive feedbacks to climate warming manifest in greater landscape and ecosystem connectivity, such as increased biomass and abundance of organisms, and enhanced biogeochemical cycling. Because several of these hypotheses were not supported, MCM5 considered the potential for increased connectivity to lead to altered ecosystem states, as opposed to simple linear increases in diversity or cycling rates. In both iterations of the project, hypotheses were based on connectivity change. Now, in MCM6, we propose that the temporal dynamics of connectivity – specifically, the magnitude, frequency, and duration of connections across the ecosystem – modulate the historical influence of material legacies on ecosystem structure and functioning.

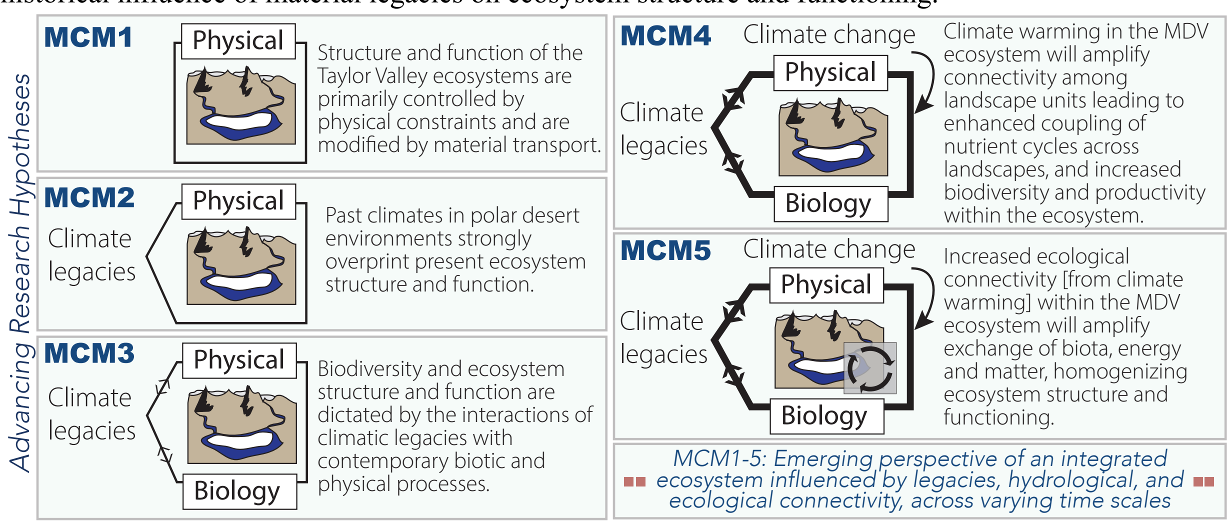

Figure 6. Historical hypotheses and conceptual figures depicting the intellectual development in MCM1-5.

2.1.1 Evolution of the MCM Conceptual Framework Model - The first three cycles of the MCM (1993-2010) focused on the physical drivers controlling the structure and functioning of this polar desert landscape, but considered long time scales associated with the legacies of geologic history and geomorphology (Fountain et al. 1999) (Fig. 6). The recognition that glacial history, landscape position, and geochemical evolution of soils, streams, and lakes influenced contemporary biogeochemical cycling and the distribution and activity of biota is still a guiding principle in MCM research (Virginia and Wall 1999, Lyons et al. 2000, Xue et al. *In review*). With this perspective, we came to view the distinct glacial, terrestrial, and aquatic landscapes as linked across geological time (Barrett et al. 2007, Welch et al. 2010), and this integrated landscape view influenced the conceptual models of MCM 4 and 5, which considered ecological connectivity among these landscapes over contemporary time scales (Gooseff et al. 2017b, **Sokol et al. 2020**). For example, the high-melt summer during the 2001-02 field season significantly altered the structure and functioning of aquatic and terrestrial ecosystems throughout Taylor and Wright Valleys (Foreman et al. 2004, Barrett et al. 2008b, Doran et al. 2010, **Gooseff et al. 2017a**). We applied a conceptual model describing how connectivity among landscapes is subject to physical and ecological feedbacks that influence the resistance and resilience of biological communities and ecosystem processes to climate-driven environmental change. Ongoing studies tested these ideas in landscape-scale experiments (§3.2.3).

During MCM6 we will examine the responses of this polar desert ecosystem to physical disturbances manifest as anomalous weather events, climate change, and through related manipulative experiments (§3.3). We will consider temporal dynamics more explicitly by considering the drivers of ecological processes across a continuum of relevant time scales (Table 3). We have developed a suite of approaches to address gaps in our current understanding of how the distinct temporal scales of abiotic drivers influence different ecological processes from the level of molecular and physiological responses of organisms to the responses of biological communities and ecosystem processes. Our objective is to examine the temporal variation over multiple scales in aquatic and terrestrial biological communities, and their contribution to ecosystem functioning in light of the changing climate conditions experienced as daily, seasonal, and inter-annual weather variation (Figs. 6, 7). Understanding natural temporal variation in biotic communities and ecosystem processes (i.e., noise) is necessary to the detection of anthropogenic climate change effects on ecosystems (i.e., signal).

Table 3. Time scales for biota and processes investigated in MCM6.

Time scale	Biota	Process	Landscape
Min-Days	Re-activation of Nostoc mats	C-cycling	Wind redistribution
Daily	Emergence from anhydrobiosis	Weather, streamflow	Active layer refreeze
Seasonal	Growth & reproduction Acclimation, dessication/cryptobiosis	Flood events	Moat thaw/freeze
Interannual	Population dynamics	Warm/wet, cool/dry	Permafrost degradation
Decadal	Acclimation	Weathering	Cryoturbation
Centennial	Adaptation	Deposition	Lake inundation
Millennial	Evolution		Lake recession

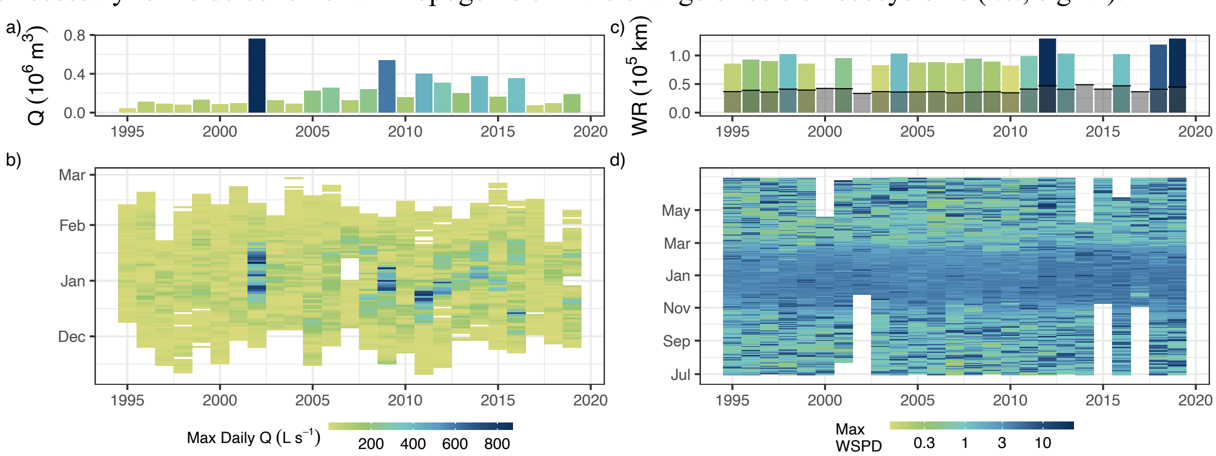

Figure 7. a) Annual total streamflow (Q) and b) maximum daily streamflow from Canada stream. c) Annual total windrun (WR), with summer (Nov-Feb) windrun highlight in grey, and d) maximum daily wind speed from the Lake Fryxell meteorological station.

2.2. Hypotheses.

2.2.1 Overarching Hypothesis - In MCM6, we consider the ecosystem consequences of temporal variability in connectivity, including connections occurring with low to high frequency, weak to strong magnitude, and short to long duration. Aeolian connectivity is often short duration (foehn storms occur for <1 day to a few days), infrequent across wide scales (~monthly), and variable in magnitude but more consistent in winter. Streamflow, in contrast, is of longer duration (weeks to months, only in summer) and varies in magnitude depending on meltwater generation (Fig. 7). During warmer summers, high connectivity can occur for several weeks in the summer between the lakes, streams, and soils through the melting of perimeter moats (Fig. 1C). Abiotic and biotic attributes of the MDV ecosystem also have predictable annual rhythm. Six months of polar night occurs every year and the light regime varies little (except for cloudiness) in the summers (Fig. 8), and air temperatures plunge far below freezing in the austral winters and rise a few degrees above freezing in the austral summers (though soil surfaces can warm to 20°C). These daily, seasonal, and annual events occur within a context of millennial-scale climate variation that includes both cooling and warming, which elicit significant responses of the mass balance of glaciers and lakes (Doran et al. 2008, Hall 2009). Punctuating these time spans and degrees of connectivity are extreme weather events, such as the flood of 2001-02, which can significantly alter the existing structure and functioning of the MDV ecosystem. However, the resident biota in these ecosystems have evolved under these extreme regimes of energy and light input. The landscape has also developed from geologic, glacial, and prior ecological processes that have left legacies of minerals, organic matter, nutrients, and widely varying exposure ages.

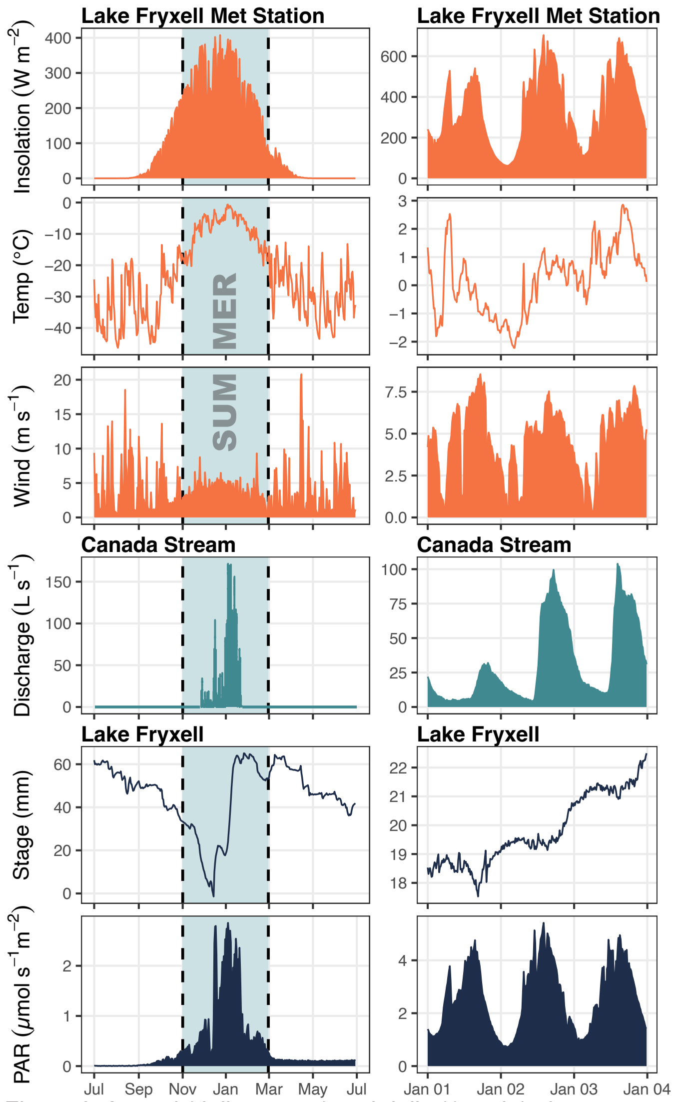

Figure 8. Annual (daily means) and daily (15-min) observations of meteorological and hydrological observations in the Lake Fryxell basin (2017-2018), including insolation (solar radiation), air temperature, wind speed, streamflow discharge, relative lake level [stage], and underwater PAR. The austral summer is highlighted by the blue ribbon.

> ***Our overarching hypothesis for MCM6 is: The structure and functioning of the MDV ecosystem is dependent upon legacies and the contemporary frequency, duration, and magnitude of ecological connectivity.***

2.2.2 Working Hypotheses - To address this overarching hypothesis and to test the dependency of these systems on the frequency, duration, and magnitude of ecological connectivity, we propose the following four working hypotheses that integrate our proposed experiments and monitoring activities (Fig. 9).

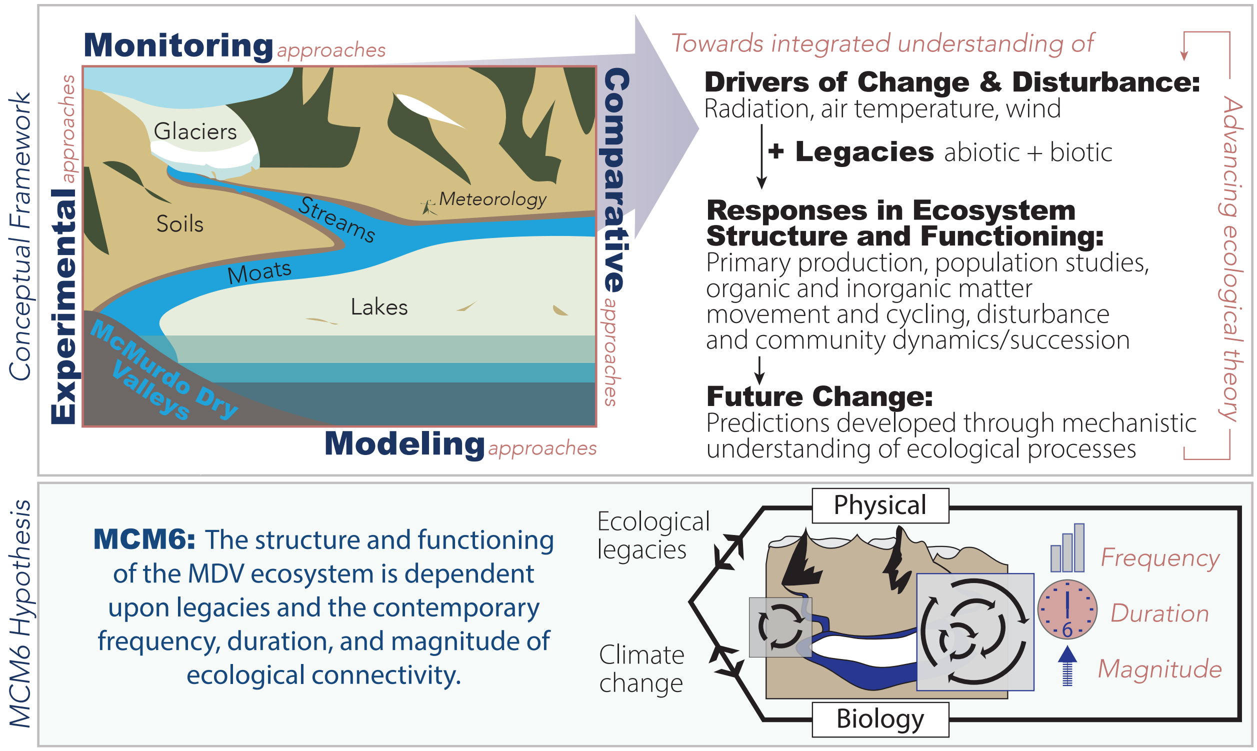

Figure 9. MCM6 conceptual framework and overarching hypothesis.

H1: The stability of the MDV ecosystem with respect to contemporary variation in abiotic drivers is determined by the responses of sentinel taxa. We define sentinel taxa as those which often exhibit high sensitivity to environmental disturbance, such that surveillance of these taxa can be used to assess ecosystem health (Table 2). For example, microbial picoeukaryotes are sentinels of Arctic Ocean warming (Freyria et al. 2021, Demory et al. 2019), while specific bacterial taxa are sentinels of warming and freshening events in the Western Antarctic Peninsula (Alcamán-Arias et al. 2021), and certain endemic diatom taxa thrive in highly variable stream flow conditions (Esposito et al. 2006, Stanish et al. 2012). Furthermore, sentinel taxa may also regulate or perform critical functions in ecosystems. For example, in the MDVs, nematode species respond to changes in water availability in predictable ways and are apex level consumers responsible for ~10% of C turnover (Barrett et al. 2008a). Similarly, several phytoplankton taxa are responsible for the vast majority of autochthonous C in MDV lakes (**Li and Morgan-Kiss 2019**, Dolhi et al. 2015) and streams (Alger et al. 1997). Thus, responses of sentinel taxa to changes in abiotic drivers can alter major ecological processes. Ecological resilience is a challenging concept to define, and to test (e.g., Carpenter et al. 2001). Our answer to Carpenter et al.'s (2001) titular question "*Resilience of what, to what?*" is to measure multiple aspects of diversity (richness, composition, functional) and ecosystem processes (gene expression, extracellular enzyme activity, respiration, and photosynthetic pigment activity) over multiple time scales. This will allow us to assess the resilience of MDV sentinel taxa to the abiotic variation associated with a changing climate, and to identify linkages between these organismal and population-level responses to resilience at an integrated community and ecosystem level. To test H1, we have developed surveillance activities of sentinel taxa in each landscape unit. *We predict that the resilience of aquatic and terrestrial ecosystems to environmental disturbance will correlate with the changes in diversity that result from sentinel taxon responses to disturbance.*

H2: The resilience of the MDV ecosystem to disturbance is dependent upon ecological legacy. In MCM2, we developed a legacy model that linked geological history and paleoclimate to contemporary ecosystem structure and functioning (Moorhead et al. 1999, Burkins et al. 2000, Lyons et al. 2000). The current spatial distribution of nutrients, organic matter, biomass and organisms is influenced by ancient physical drivers. Some of these legacy drivers include soil salt accumulations and valley-filling paleolake deposits of ancient organic matter in soils, creating gradients of nitrogen and phosphorus availability among the different MDV basins that constrains biological activity (Barrett et al. 2007, 2010, Zeglin et al. 2009). Other legacies include the chemoclines and biomass accumulation of the highly stratified lakes (Lyons et al. 1998) and glacier mass-balance, which influences stream flow (Fountain et al. 1999). These ecological legacies, which arose and have persisted since the Neogene (e.g., Halberstadt et al. 2022), overprint the different MDV landscape features and are powerful drivers of contemporary ecosystem structure and functioning. Testing hypotheses about how MDV ecosystems respond to contemporary climate variability (H1, H3, H4) requires that our observations be interpreted in the context of legacies persisting, despite observed contemporary changes in physical and biological connectivity. For example, even massive changes in contemporary hydrological connectivity may be insufficient to alter legacy drivers of diversity and distribution, and subsequent ecosystem structure and functioning. *Thus, H2 predicts that the persistent effects of ecological legacy play a key role in maintaining ecosystem resilience to contemporary climate-driven changes. Importantly, H2 distinguishes ecological legacy from the contemporary drivers of ecosystem responses of the experiments and observations that are explicitly tested by H1, H3, and H4.*

H3: Carryover of water, organic matter, and nutrients through periods of low connectivity maintains biological activity and community stability. In desert and drought-prone ecosystems, biological activity can rebound quickly following water pulses after a dry period (Schimel et al. 2018, Collins et al. 2014, Evans and Wallenstein 2012, Osburn et al. 2021). In the MDVs, we know that the availability of resources for resident biota vary over multiple time scales, with contrasting dynamics in soil, stream, and lake landscape units. Most obviously, hydrologic connectivity to streams and soils is cut off through the winter months when glacial meltwater is no longer generated, promoting the desiccation of microbial mats (McKnight et al. 1999), and soil biota suspend biological activities until favorable conditions return the next summer. However, shorter durations of hydrologic disconnection also occur in summer months during periods of lower insolation (Wlostowski et al. 2016, Gooseff et al. 2017b) when soil water freezes (Wlostowski et al. 2018a). In soils, over 50% of the metazoan community exist in anhydrobiotic states even during the summer months, but remain capable of rapid revival upon pulses of rehydration (Treonis et al. 2000, Ball et al. 2009). During daily and weekly periods of low water availability and reduced resource influx, biological activity must rely on local nutrients, organic matter, and water. In contrast, lake biota are not water-limited, but do rely on PAR and an influx of limiting nutrients from streams and wind-borne dust. Intermittent aeolian connectivity across landscape units persists through periods of hydrologic disconnectivity, supporting local resource influx (Lancaster 2002, **Diaz et al. 2018**). Overall, *we predict that the amount of surplus resources accumulated at a locality are proportional to the level of biological activity maintained through periods of disconnection, and to the level of biological activity that is achieved upon reconnection*. We will evaluate this hypothesis in MDV streams, soils, and lakes both by monitoring biological activity at a higher temporal resolution during focused times spanning disconnection and reconnection periods, and by experimentally altering the magnitude, frequency, and duration of resource supply or biologically favorable conditions.

H4: Changes in disturbance magnitude, frequency, and/or duration disrupt the annual reset of major ecological cycles following the polar night. Winter conditions are rapidly changing in polar and temperate ecosystems, particularly those with significant seasonal snow and ice cover (Obryk et al. 2016, Hampton et al. 2017, Sharma et al. 2019). As a result, changing light, temperature extremes, snowfall, wind, and timing of these conditions can have significant and lasting influences over biotic communities and ecosystem processes during the subsequent season (Schmidt et al. 2007, Ernakovich et al. 2014). The polar night in the MDVs is a period of 6 months of darkness (Fig. 8) which is experienced differently by communities across the landscape. In soil and stream habitats, communities and are presumably dormant at the persistent <−40º C temperatures. Lake moats freeze from the top down, resulting in a gradient from shallow moat constituents that freeze prior to loss of PAR to deep moat constituents that only freeze after months immersed in darkness. Liquid water persists year-round in the limnetic zones, while light-driven primary production ceases during the winter, representing a period of poorly understood heterotrophic and chemolithotrophic processes.

We propose an extended field season to understand how environmental disturbance influences annual reset or resumption of ecological processes (e.g., primary productivity, decomposition, predation) following the polar night. Specifically, events that occur during the fall shut-down (Kong et al. 2012a, 2012b, 2014) impact both winter activity and the state of the ecosystem at the beginning of the spring start-up (Lizotte et al. 1996, Lizotte and Priscu 1998). To fully address H4, which focuses on annual ecological cycles and carryover through the polar night, we must both develop robust baselines for annual ecological and hydrological cycles beyond the traditional Antarctic field deployment season and test the influence of disturbances on ecosystem functioning and recovery into the fall/winter. To this end, we propose to conduct extended field work during the 2026-27 season into April of 2027 (i.e., capturing the polar night transition), as the traditional Antarctic field season is completed before the shutdown of hydrological activity in the MDVs, which is known to occur in March (Wlostowski et al. 2016). It is unknown, however, how these ecosystems typically “shut down” during this transition into polar night, which requires detailed monitoring to understand annual ecological cycles and their relation to environmental variables. Experiments conducted during the extended season will directly address H4 by examining how processes during seasonal shutdown propagate through the polar night, into the start of the following summer. We will extend monitoring and experiments into a late-summer and early-autumn shoulder season and we will further test H4 by conducting manipulative field and laboratory experiments, remote sensing, and year-round automated sampling. *We predict that environmental disturbances from the previous season that significantly impact the communities will disrupt natural processes which occur during the polar night.*

3 RESEARCH PLAN

Our four working hypotheses directly address the contributions of landscape legacy and the role that the frequency, duration, and magnitude of connectivity have on the MDV ecosystem. We will test these working hypotheses using a combination of ongoing and new monitoring activities and experiments (Fig. 10). Monitoring activities include the regular monitoring and sampling of physical and ecological variables using a mix of automated and manual approaches. Experiments include physical, chemical, and thermal manipulations to understand the response and potential recovery of the ecosystem following disturbance. *These collective activities are integrative, rigorous, and are bolstered by the 30+ years of successful implementation of similar activities by former and current members of the MCM team.*

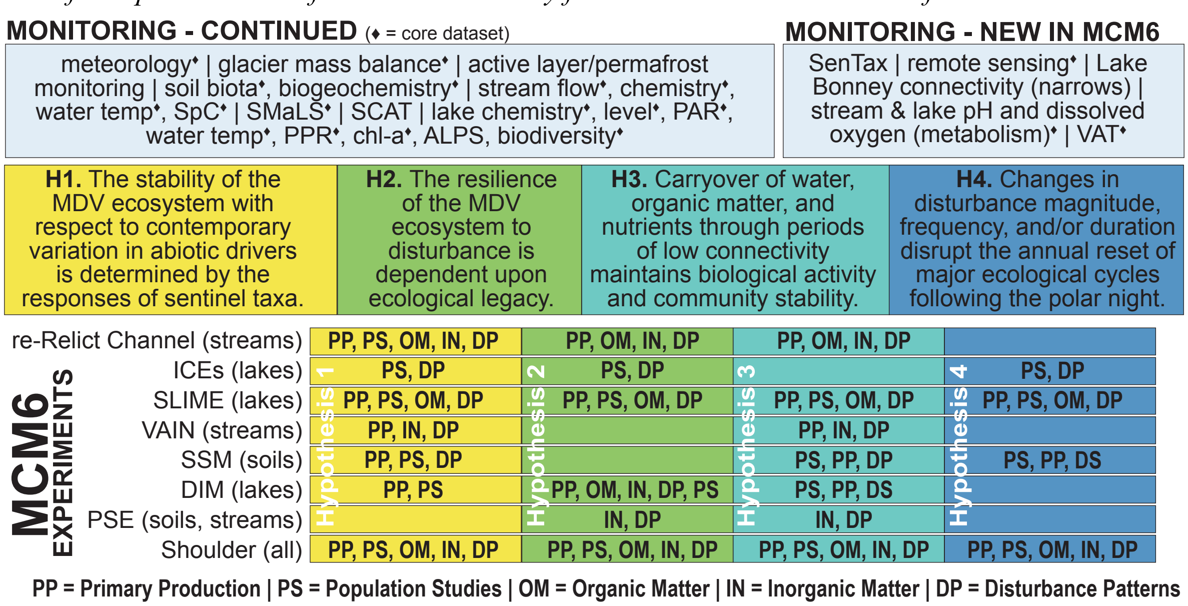

Figure 10. Relationships between continued and new long-term monitoring, experimental activities and our working hypotheses in MCM6. All experiments are mapped to hypotheses and LTER core areas of research.

Most field work in the MDV has occurred between the months of October and February when temperatures and both ecological and hydrological activity are generally greatest. However, much less is known about the nature of landscape processes and their influence on both the short- and long-term recovery of ecological communities in response to disturbance during the shoulder seasons when the environment is undergoing a relatively rapid transition. Previous work conducted during the transition from austral winter to summer (Sep-Dec) has reported on the onset of chlorophyll accumulation and PPR (Lizotte et al. 1996) as well as the timing of bacterial production (Takacs and Priscu 1998) in Lake Bonney. In studies conducted during the transition from austral summer to winter (Feb-Apr), phytoplankton dynamics in Lake Bonney exhibited temporal variation related to the trophic status of the organisms. For example, photosynthetic genes of chlorophytes were strongly correlated with light availability while the gene expression of mixotrophic haptophyte and cryptophyte populations remained high even after underwater light levels were below detection limits (Morgan-Kiss et al. 2016, Kong et al. 2012a, 2014). *While these studies capture the normal operating range of seasonal trends, we seek to determine how these transitions to and from the polar night influence ecosystem response to disturbance.*

3.1 Continuing and New Long-Term Monitoring.
Infrastructure improvements (hereafter FSML upgrades) will take place in Year 6 of MCM5 via a recent NSF Biological Field Stations and Marine Laboratories (FSML) grant to PIs Doran, Brown, and Gooseff, and are discussed throughout the text below.

3.1.1 Meteorology [Doran, Dugan] - The MDV Climate Monitoring Network (Fig. 1) consists of 14 meteorological stations, including the longest-running Lake Hoare Station that was established in 1986. Stations are situated on both soils and glaciers, with standard measurements of air temperature, relative humidity, wind speed and direction, and incoming and outgoing solar radiation (total flux). Non-glacier stations additionally measure soil temperature at 0, 5, and 10 cm depths, as well as PAR, and an additional subset of stations measure precipitation or snow depth. Additional measurements depending on location include: longwave radiation, air temperature at 1 m (glacial stations), wind speed/direction at 1 m, barometric pressure, soil temperature and moisture. Measurements are made every 30 seconds and averaged every 15 minutes using Campbell Scientific dataloggers. Data are saved on memory cards and telemetered in near-real-time via Iridium satellite. FSML improvements will include upgrading all dataloggers, increasing onboard memory, adding weighing bucket precipitation gages, augmenting barometric pressure measurements, and adding soil moisture, temperature, and conductivity sensors. *The meteorological record supports estimates of surface energy balance to quantify snow/ice melt and aeolian redistribution of materials, directly informing physical connectivity spatiotemporal patterns.*

3.1.2 Glaciers, Active Layer, and Permafrost [Gooseff, Salvatore] - Our long-term (since 1993) monitoring of several glaciers (Taylor Valley: Commonwealth; Canada; Howard; and Taylor Glaciers; Miers Valley: Adams Glacier) has focused on glacier mass balance with measurement locations distributed across each glacier to track accumulation at higher elevations and provide an estimate of net ablation at lower elevations. The glaciers represent a hydrologic legacy and provide contemporary sources of meltwater for hydrologic connectivity. *Combined with the streamflow records, these data provide direct quantification of glacier-stream connectivity (Bergstrom et al. 2021)*.

We maintain 5 active layer monitoring stations (ALMSs) equipped with temperature, soil moisture, and salinity sensors. Each station has 4-6 sensor 'chains' allowing profiles to be obtained through the active layer and into permafrost. Two ALMSs are located on streams (Green Creek, Von Guerard Stream), and have sensors installed through active layer from the thalweg out to the shoreline and dry soil beyond. Two ALMSs are similarly deployed on water tracks (zero-order drainages of snow/ice melt that rarely have surface flow). The 5th ALMS is deployed in dry soil near a long-term soil experimental site on the south shore of FRX. Multispectral UAV overflights and ground-based validation will be used to improve our correlation between surface albedo and soil moisture (§3.1.9), and to *estimate the bidirectional fluxes of heat and moisture across the MDV landscape*.

3.1.3 Soils [Adams, Barrett, Salvatore, Zeglin, Diaz] - An elevational transect (established in Taylor Valley in 1993 and extended to Miers and Garwood valleys in 2012, Powers et al. 1998, Porazinska et al. 2002, **Andriuzzi et al. 2018a**) and control plots of former experiments such as the biotic effects experiment (established 1999, Simmons et al. 2009) are at the core of MCM soil monitoring in which invertebrate diversity and community structure, surface chl-a, and soil chemistry are measured annually. Continuing monitoring plots related to lake-level rise include invertebrate community structure of soils, moat, and benthic mats and sediments (§3.2.3). We will also continue to characterize the diversity and functioning of soil microbial eukaryotes, and will expand our monitoring to use satellite- and ground-based spectral techniques (§3.1.9) to add to our regular suite of data collection. New approaches to testing hypotheses of ecosystem resistance and resilience will use previously established experimental and control plots that received manipulative water, nutrient and temperature treatments (§3.2.1). *These data will allow us to test the soil-related components of H1-H4.*

3.1.4 Streams [Gooseff, Diaz, Kohler, Salvatore, Zeglin]

Stream Flow and Water Chemistry - We currently operate stream gages at 17 locations in three valleys (Fig. 1). The longest streamflow record is from the Onyx River in Wright Valley, which was established in 1968 (by the New Zealand Antarctic Programme; MCM LTER took over in 1993). Other gauging records began in or after 1993 at the start of the MCM LTER. All stream gages measure stage, SpC, and temperature. In the 2022-23 field season, FSML upgrades at each station will include pH and dissolved oxygen (DO) sensors, upgraded dataloggers, and greater telemetry coverage. From DO records we will estimate whole stream metabolism as a new stream data product. We currently provide near-real-time telemetered data on our project webpage from seven gages (*see Data Management Plan*). Priority has been given to the most distal gages so that we can make real time decisions about site visits. Gages are maintained each field each season, and discharge measurements are made to populate and/or re-define rating curves. Stream water samples at all gaging stations are collected throughout the flow season, analyzed for DOC, nutrients (NO_3^-, NO_2, SRP, and NH_4^+), major ions, alkalinity, and Si. *These streamflow and chemistry records continue to help us define hydrologic connectivity dynamics in space and time.*

Stream Mat Long-term Surveys (SMaLS) - As MDV stream ecosystems continue to experience and transmit the consequences of hydrological change, it is essential to continue long-term collection of data on microbial mat standing stocks and diversity. We will continue and expand long-term monitoring of microbial mat chl-a and biomass at streams with the longest records. The ten continuing long-term core transect sites will include locations representing the range of legacy templates and hydrological flow regimes that exist across the MDVs, with four sites in the Fryxell basin, and two sites each in the Taylor, Wright, and Miers Valleys (Fig. 1). Monitoring at these selected locations will expand to include functional assays, specifically stream mat extracellular enzyme activity (EEA) rates, the ratios of which reflect the biological limitation of N and P as constrained by intra-basin geological differences, which will provide a test of H2 in the streams (Barrett et al. 2008a, Zeglin et al. 2009). Taxonomic diversity measurements of diatom morphospecies (Esposito et al. 2006, Stanish et al. 2012, Kohler et al. 2016) and bacterial, archaeal, and eukaryal 16S and 18S rRNA gene sequence variants (Zeglin et al. 2011, Van Horn et al. 2016) will also be included formally in the long-term data collection, to address H1. Diatom morphospecies identification is well-established through prior research in the MDVs and will continue through sample preparation in the INSTAAR Phycology Lab and analysis by Dr. Tyler Kohler. Together, we will conduct one targeted study directly comparing diatom morphospecies taxonomic identification with multilocus sequencing (18S rRNA, *rbc*L, and *psb*C (CPL); Ruck and Theriot 2011) to determine whether diatom gene sequence variation can be as direct an indicator of flow regime as morphospecies identification, and thus establish continuity between past, present, and future diatom diversity surveys. Towards greater integration among landscape units, all methods will be standardized with lake benthic mat sampling (§3.2.3). Further, we will use satellite- and UAV-derived multispectral imagery and derived data products (§3.1.9) to scale up biomass measurements to estimate annual carbon stocks in the MDVs (Salvatore et al. 2021a). The continued field monitoring efforts will also be used to validate remote datasets and will allow us to extrapolate the documented fluctuations of primary production in wet and dry years (**Gooseff et al. 2017a**) from the local scale to the basin scale, and to learn how the magnitude of carbon available to the whole ecosystem is affected by temporal changes in hydrological connectivity, supporting all working hypotheses. *These data will directly facilitate quantitation of spatiotemporal stream ecosystem structure and functioning for H1-H4.*

3.1.5 Valley Aeolian Transect (VAT) [Diaz, Dugan, Salvatore] - Aeolian transport in the MDVs is an important vector for sediment, nutrient, and biological connectivity (Fig. 5) (e.g., Lancaster 2002, Nkem et al. 2006, Šabacká et al. 2012, Deuerling et al. 2014, **Diaz et al. 2018**, Schulte et al. 2022). While we have several years of annual and seasonal aeolian material collection (see **Diaz et al. 2018**), we are now formally incorporating aeolian collection into our monitoring suite. Passive aeolian sediment traps will be installed at six locations in Taylor Valley (Fig. 1) at two heights: ~20 cm above the surface to capture short distance saltation, and at 1.5 m which is above the saltation threshold (Lancaster 2002). The traps will be modeled after the U.S. Geological Survey long-term dust modeling (Reheis and Kihl 1995) where Bundt pans are outfitted with a mesh screen and glass marbles. Aeolian collectors will be co-located near existing meteorological stations to couple sediment flux and chemistry with wind speeds and foehn events. Collectors will be sampled once in the early summer season (November) and at the end of the season (February) to capture winter and summer signals. Seasonal differences in water-soluble nutrients exist throughout Taylor Valley (**Diaz et al. 2018**). The aeolian material will be analyzed for amount, grain size (where appropriate mass has been collected), hyperspectral VNIR reflectance signatures, water-soluble salts and nutrients (nitrate and SRP), and total organic carbon (TOC). Amplicon (16S and 18S rRNA gene) sequencing will be done on material once per year to determine which organisms are transported by winds. *This new monitoring will directly address the LTER core areas of movement of inorganic matter, movement of organic matter, and possible population studies if the sequencing yields enough counts*.

3.1.6 Stream Connectivity Aeolian Transects (SCAT) [Gooseff, Barrett, Adams, Diaz] - We initiated the SCAT monitoring activity in MCM5 to quantify the aeolian deposition of organic and inorganic material into streams. Recent findings from examination of aeolian sediment (Nkem et al. 2006, Šabacká et al. 2012, **Diaz et al. 2018**, Shulte et al. 2022) and the presence of algal mat material on MDV glaciers and in cryoconite holes (Bagshaw et al. 2011, Porazinska et al. 2004) indicate that there is significant movement of organisms across the landscape. The incised nature of most MDV stream channels makes them an ideal location for deposition. Eight passive aeolian samplers are deployed along each of 6 transects along Von Guerard Stream - three incised locations, three non-incised locations. Samplers are affixed to posts ~20 cm above the ground surface. Transects include distal samplers located ~5 m from the edge of the channel, another ~1 m from the edge of the channel, and then two in the channel bottom toward the active wetted portion. This same distribution is mirrored on the other side of the active portion of the channel. Samplers were deployed in January 2018 and only one set of samples was collected prior to the pandemic impacted seasons. Collected and analyzed data include total mass, ash-free dry mass and nutrient content. *These data will reveal how much allochthonous material is contributed to stream ecosystems, revising our conceptual model of these streams and addressing H3 and H4*.

3.1.7 Lakes [Doran, Dugan, Morgan-Kiss, Hawes, Mackey, Salvatore, Takacs-Vesbach] - Measurements in the water columns of ELB, WLB, FRX, and Hoare will continue through a combination of the regular profiling measurements and sample collection ("limno runs"), year-round high-frequency measurements using lake monitoring platforms consisting of automated sensor arrays that measure underwater PAR, water temperature, and pressure, as well as dissolved oxygen and pH from FSML upgrades, and manual lake level measurements. Limno run frequency will be increased by focusing our efforts more intensively on one lake each year, although core measurements will still be made at the other lakes. We also plan to revise our sampling by collecting some key measurements at only critical depths and cutting other measurements that have proven to be less informative (e.g., bacterial cell counts) and replacing them with proxies for biomass such as DNA quantification (Reynebeau 2021). Productivity and community composition dynamics are most pronounced from December to January but remain poorly resolved due to sampling at frequencies greater than the doubling times of the organisms. Thus, we will sample every two weeks from November to January (5 vs. 3 samplings) to allow for higher frequency monitoring during the summer months. *We will test H1 by providing high resolution data of the activity and abundance of sentinel lake taxa (§3.1.8) during the growing season. These activities will also test H4 by providing detailed information on development of primary production in the summer, fall shut-down, and spring start-up*. The ELB and WLB will be the focus in year 1 and 2, respectively, followed by FRX in year 3. The shoulder season in year 4 (§3.3.5) will be an opportunity to capture the summer-fall-winter transition at FRX, and in year 5 we will focus on HOR.

Narrows Connectivity: We will be establishing a blue box (mooring) at the Lake Bonney Narrows to quantify the connectivity between WLB and ELB via sporadic flows of dense WLB water across the lake sill that separates the two lakes (Fig. 11). These efforts are in response to the MCM5 site review recommendation for "*quantification of the connectivity of subsurface groundwater (Blood Falls; Lake Bonney) flow to lake dynamics and communities*," and supports our overarching efforts to quantify the role of connectivity on shaping ecosystem structure and functioning. The surface blue box will be wired to conductivity and temperature sensors at the deepest location in the narrows to capture "spill-over events" from WLB that are caused by the combination of externally forced wind-driven barotropic water movements (Spigel et al. 2018, Castendyk et al. 2022) and internal increases in WLB water volume due to episodic inputs of subglacial meltwater from Taylor Glacier into the WLB hypolimnion (Lawrence et al. 2020). We will measure major ion concentrations and conduct 16S and 18S rRNA gene sequencing using higher vertical resolution limnological run measurements to track the flux of solutes and biota between the lakes. We will focus these measurements near the sill depth on the WLB side and at the depth of neutral buoyancy for the overflowing water on the ELB side. *These measurements will address H3.*

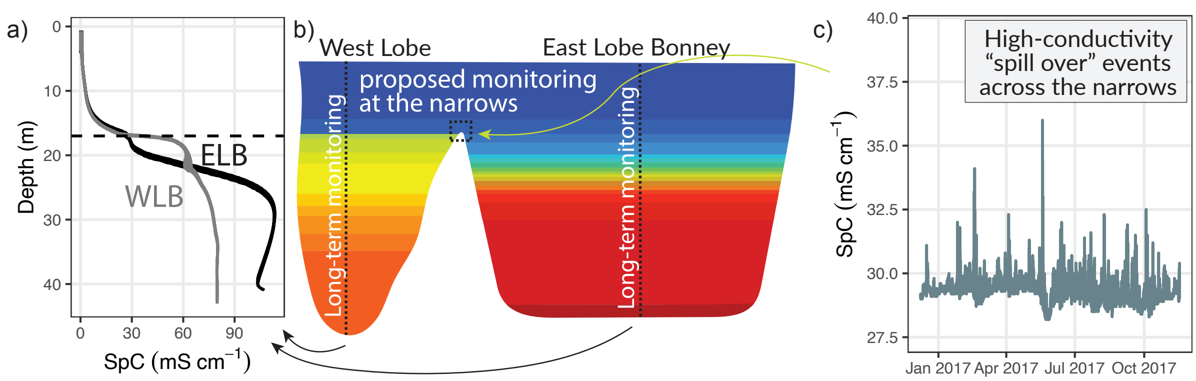

Figure 11. a) Depth profiles and b) spatial extent of specific conductance (SpC) in West Lobe Bonney (WLB) and East Lobe Boney (ELB). b) Location of long-term monitoring and new proposed monitoring location at the narrows. c) A short-term deployment of a high-frequency logger show spikes in conductance when water spills over from WLB to ELB across the narrows.

Autonomous Lake Profiling System (ALPS): We regularly deploy two ALPS that are composed of three McLane Laboratories instruments: one Phytoplankton Sampler (PPS), one Remote Access Sampler (RAS), and a modified Ice Tethered Profiler (ITP). The PPS and RAS are programmed to collect and preserve lake water samples from the primary productivity maxima of lakes for community and biogeochemical analysis at 22-day intervals through the year. The ITP, which includes a CTD, a spectrofluorometer (BBE FluoroProbe), and a PAR sensor, profiles 10-20 m around the PPS and RAS (water density and the highly reducing water at depth prevents whole lake profiles). ALPS has been deployed for multiple years in ELB and WLB, and in HOR during 2019-2020; however, due to Covid-19, equipment and samples were not recovered until 2022. We will deploy ALPS in MCM6 in FRX and HOR in years 1-3. We are also planning to use ALPS for *in situ* LICE (§3.2.4) in year 4.

Data from the FluoroProbe during the 2013-2014 deployment showed an unexpected rise in winter chl-a levels in both ELB and WLB followed by a precipitous decline in late winter (Fig. 4; **Patriarche et al. 2021**). The rise in chl-a was associated with a "mixed" population dominated by a mixotrophic haptophyte, *Isochrysis* (Dolhi et al. 2015, **Li and Morgan-Kiss 2019**). 18S rRNA gene sequencing revealed that a fungal parasite, Cryptomycota, and a predatory protist (*Spumella*) were more abundant during winter. Oceanospiralles also increased in winter. Bacteria and eukaryote community dynamics were correlated with *in situ* temperature and PAR.

ALPS activities directly test MCM6 hypotheses H1 and H4. H1 predicts that key taxa occupying critical roles in the MDV lake food webs will exhibit distinct temporal patterns of abundance, activity and functional roles. H4 predicts that climate-related long-term changes in chlorophyte populations will lead to greater chlorophyte biomass during the following growing season. Community diversity was lowest in winter, but chl-a peaks associated with an increase in mixotrophic organisms were related to bacterioplankton community composition changes, suggesting that interactions among bacteria and mixotrophic grazers are significant in winter.

3.1.8 Sentinel Taxa (SenTax) [Morgan-Kiss, Barrett, Adams, Takacs-Vesbach, Zeglin, Mackey, Salvatore] - This new monitoring effort will formally identify sentinel taxa in the different landscape components and ecosystems through integration with new and continuing MCM6 activities. Within soils, *Scottnema lindsayae* is the most abundant and widely distributed sentinel taxon, especially in the typically dry soils that prevail in the MDVs. In contrast, *Eudorylaimus* spp. and *Plectus* spp. occupy saturated to intermittently wet soil and sediment environments near streams, lakes, and snowpacks and exhibit population change following interannual variation in water activity (Treonis et al. 1999, Ayres et al. 2008, Barrett et al. 2008b, **Gooseff et al. 2017a**, **Andriuzzi et al. 2018a**). We will continue to examine community dynamics of soil invertebrates in long-term monitoring plots (§3.2.1) and in our new seasonal soil manipulations experiment (§3.3.1). Stream mat surveys (§3.1.4) will augment regular monitoring with laboratory characterization of the physiological ranges for dominant stream benthic mat primary producers (*Nostoc* spp., Oscillatorian cyanobacteria, and a chlorophyte *Prasiola* spp.) (Alger et al. 1997, McKnight et al. 1998), and the diatom species (endemics *Hantzia hyperaustralis* and *Luticola permuticopsis*, vs. comsopolitan *H. amphioxys*) that indicate differential flow regimes (Esposito et al. 2006, Stanish et al. 2012). Moats are a transitional environment from terrestrial and stream landscape units to the main lake body under perennial ice (§3.2.3). Under the perennial ice cover, a core group of sentinel phytoplankton taxa (*Chlamydomonas, Geminigera cryophila, Isochyrsis*) play critical roles in C cycling in the MDV lakes (Kong et al. 2012b, 2014, Dolhi et al. 2015, Bielewicz et al. 2011, Li et al. 2019, **Li and Morgan-Kiss 2019, Patriarche et al. 2021**). Benthic microbial mats are dominated by distinct cyanobacterial assemblages that vary with depth (Dillon et al. 2020a, 2020b), such as surficial biofilm dominated by *Phormidium pseudopriestleyi* (Jungblut et al. 2016) that generate seasonal oxic microenvironments in euxinic waters below the chemocline (Lumian et al. 2021).

In addition to the field collections described above, we will use archived amplicon sequencing data and archived (frozen) samples from previous years to analyze long-term spatial and temporal trends in key taxa. *Using field data from the MCM monitoring and experiments, and lab manipulation experiments on isolates, we will test H1 by examining the resistance and resilience of individual sentinel taxa to new changes in climate-driven environmental regimes (e.g., light, salinity, temperature, water, nutrients).* The lab-controlled experiments will provide opportunities for additional undergraduate and graduate students to develop focused research questions on sentinel taxa that are not logistically feasible in the field. *For example, responses of sentinel taxa to mimicked winter conditions will test H4.*

3.1.9 Remote Sensing [Salvatore] - In MCM6, we will expand the spatial and temporal capabilities of our monitoring activities using a range of remote sensing technologies. This builds on previous research that demonstrated both the utility and accuracy of these techniques for characterizing the ecological properties of these unique landscapes (e.g., Levy et al. 2011, Salvatore et al. 2014, 2021a, 2021b, Power et al. 2020). Regional Landsat-derived products will be generated each spring (~October), summer, (~January), and fall (~March) for the entire MDVs to characterize seasonal variations in surface properties. High-resolution products will be generated for all of Taylor Valley and the major MDV lakes in other valleys using multispectral WorldView data. We have also identified several additional ground validation targets that will be used to improve our ability to atmospherically correct both Landsat and WorldView data (Fig. 12; e.g., Salvatore et al. 2021a).

Seasonal Landsat products include: (1) calibrated surface reflectance using data collected from ground validation targets, (2) topographically corrected surface albedo (Salvatore et al. 2021b) at PAR wavelengths (400 - 700 nm), (3) volumetric water content (VWC) estimates using lab- and field-derived calibration curves (Salvatore et al. 2021b), (4) Normalized Difference Vegetation Index (NDVI; Tucker 1979); (5) results from landscape-scale spectral mixture analyses (SMA; Adams and Gillespie 2006) that uses field-derived spectral endmembers to model the surface abundance of each phase at each image pixel; (6) binary maps of snow/ice distribution (modeled at > 50% abundance from SMA results), and (7) biomass estimates at each pixel using the methods of Salvatore et al. (2021a). All WorldView data collected over Taylor Valley will be similarly processed, analyzed, and archived. At each of the major MDV lakes monitored in MCM6, WorldView data will be used to generate vector maps of the (8) shoreline, (9) moat ice/open water contact, and (10) lake moat/perennial ice contact to monitor changes over time. Lastly, these data will also be unmixed using SMA to (11) model the spectral contributions from snow, blue ice, open water, sediments, and organic contributions to the ice and moats. We will ensure all products are georeferenced to the most current GIS basemap used by the Polar Geospatial Center (see *Letter of Collaboration*). New ground validation targets will be defined and characterized using a gridded spectral ground validation approach (Fig. 13) (Salvatore et al. 2021a). Grids of 100 x 100 m will be established in Taylor Valley and near meteorological stations at Lake Vanda, Lake Vida, Lake Brownworth, and in Miers Valley. Additional 25 x 25 m grids will be established in Taylor Valley to supplement the calibration of WorldView data. *These remote monitoring activities directly address several LTER core areas and all of the MCM6 hypotheses, as they will be able to track both hydrological and ecological changes over time in response to natural and human-caused disturbances (Fig. 10).*

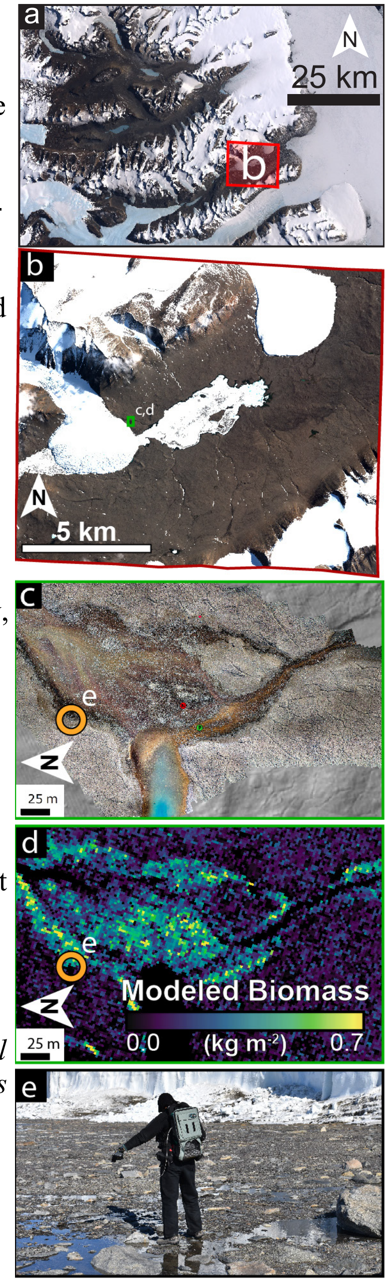

Figure 12. Scales of remote sensing efforts in MCM6. a) Landscape-scale using Landsat multispectral data. Basemap is the Landsat Image Mosaic of Antarctica (LIMA). b) Local-scale using WorldView multispectral data. Image ID is 10400100485D6900. c) Ultra high-resolution multispectral uncrewed aerial vehicle (UAV) data (from Levy et al. 2020). d) Example of ultra high-resolution data product to be generated and made publicly available. e) Ground-based spectral validation and experimental data collection.

3.2 Continuing Long-Term Experiments.

3.2.1 Biotic Effects Experiment (BEE) and Soil Stoichiometry Experiment (SSE) [Adams, Barrett] - BBE and SSE were initiated in 1999 and 2007, respectively, to address questions about how changes in physical conditions and nutrient availability influenced soil communities (Simmons et al. 2009, **Aanderud et al. 2018**). Previous work has shown that metazoan communities are resistant to low levels of mechanical disturbance from human foot traffic (Ayres et al. 2008), but that temperature (Simmons et al. 2009), increased water availability (Barrett et al. 2008b, **Andriuzzi et al. 2018a,** 2018b) and nutrient additions (Ball et al. 2018, **Aanderud et al. 2018**) significantly alter community structure and functioning. A soil colonization experiment also demonstrated that viable microbial communities and nematodes are capable of recolonizing heat-sterilized soils within 12 years (Adams et al. unpublished). These results suggest that communities are initially susceptible, but ultimately resilient, to anticipated levels of climatic and

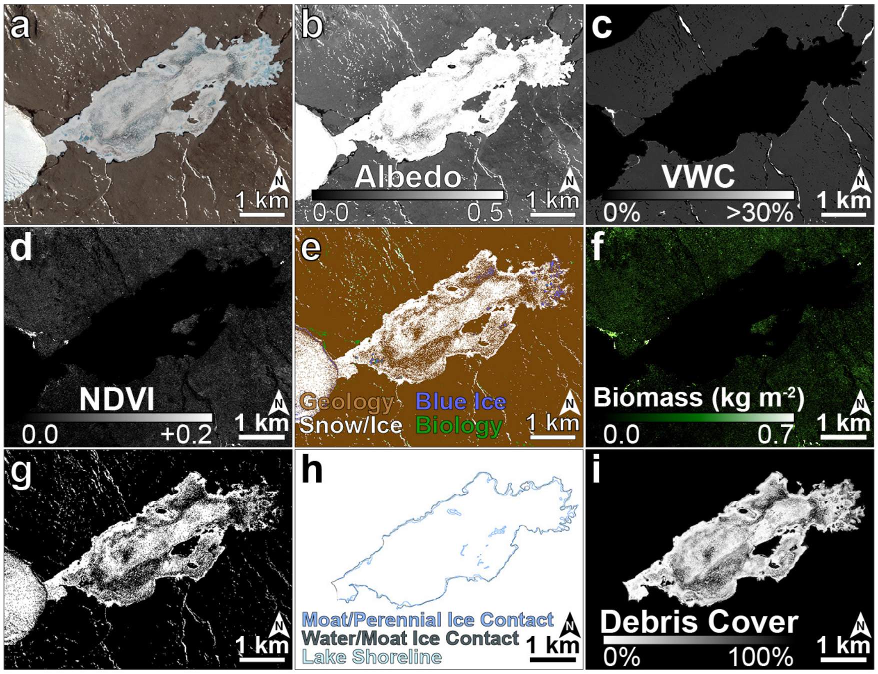

Figure 13. Remote sensing-derived data products in MCM6. (a) Calibrated multispectral surface reflectance; (b) topographically corrected surface albedo at PAR wavelengths; (c) volumetric water content (VWC); (d) Normalized Difference Vegetation Index (NDVI); (e) results of spectral mixture analysis (SMA); (f) modeled biomass abundance; (g) snow/ice distribution; (h) vector maps of lake margins and contacts; and (i) SMA results of lake ice and surface cover. All data derived from WorldView-2 image 1030010089D13500, ©DigitalGlobe, Inc.

human disturbances. In MCM6, we will repurpose these experiments to examine the resilience of soil communities to experimental treatments of warming, wetting, nutrient enrichment, and physical disturbance (using marked walkways between experimental blocks) over decadal time scales. These experiments were intentionally established on soils derived from glacial tills with distinct glacial history and N:P ratios and thus provide an opportunity to examine how ecological processes like succession and resilience are modulated by ecological legacies. *We will use our long-term data and new samples collected from the old BEE and SEE experimental plots in year two of MCM6 to test H1-3.*

3.2.2 Relict Channel Re-Activation Experiment (re-Relict) [Gooseff, Zeglin, Diaz, Kohler, Salvatore] - During MCM1 (1995), a Relict Channel (RC) that had been dry for three decades was reconnected to source flow via an upstream diversion (McKnight et al. 2007). Shortly after rewetting, dried mats were revived and N-fixation was detectable within 24 hours, and primary productivity in the RC was almost twice as high as in a parallel reference stream; furthermore, RC stream water supported significantly higher productivity of mats originating from both the RC and Canada Stream, suggesting that higher levels of nutrients (nitrate, SRP, and micronutrients) were mobilized from the RC (McKnight et al. 2007). Over the course of MCM1-5, algal mats, diatom communities, and mat N and C have been monitored in the RC, with results suggesting that ecological legacy and contemporary changes in abiotic factors support the rapid and elevated response of biota following re-wetting of a decades-dry stream channel (Stanish et al. 2012, Andriuzzi et al. 2018b**, Kohler et al. 2018**). However, in the nearly 30 years since the initiation of this experiment, automated sensors, molecular sequencing, analytical chemistry, and remote sensing capabilities have improved significantly, and we can now effectively surveil the drying and reactivation of this channel at greater spatial and temporal resolution.

To understand whether changes in hydrologic connectivity on different time scales have a similar magnitude of influence on linked abiotic and biotic responses, we will modify flow through the RC over multiple years in MCM6 and monitor ecosystem changes in the RC and reference stream channels. A constructed sandbag retaining wall currently maintains the RC, so we can manipulate this diversion with minimal added disturbance, by returning RC flow to the main contributing channel. Flow in the RC will be ceased for Year 2, and re-wetting and monitoring of the channel will occur in Year 3; the manipulation will be repeated for a two-year dry period (Years 4 and 5) and reactivation measured in Year 6. We will repurpose active layer monitoring stations to measure hyporheic moisture, temperature, and conductivity, and will also implement the suite of stream monitoring metrics described in §3.1.4. To compare daily, seasonal, and multi-annual flow pulse responses, we will collect similar measurements at the reference sites Canada Stream (which has an historically low-variability flow regime) and Von Guerard Stream (high-variability flow regime) (Fig. 14a) during Years 3 and 6 for higher-frequency daily and intra-seasonal measurements (similar to **Singley et al. 2021**). UAV multispectral data will be acquired along the length of the RC before and after each manipulation at several timescales to characterize changes in soil moisture over time as well as the behavior of photosynthetically active communities following methods of Salvatore et al. (2021a, 2021b). To evaluate H1, we predict that *Nostoc* (black) and Oscillatorian (orange) mats respond differently to the magnitude of flow change (**Gooseff et al. 2017a**) (Fig. 15) due to contrasting physiological tolerance to flow and nutrient limitation, while the indicator diatom taxa that prefer a more variable hydrological regime (Stanish et al. 2012) will rebound following reversion to the pre-existing flow conditions (H2). To evaluate H3, we predict that nutrients and organic matter will be stored within the stream channel sediments during drying and will become readily available and rapidly consumed upon re-wetting. *This experiment tests how stream biology and water chemistry are resilient to prolonged manipulations in flow, in concert with H1-3.*

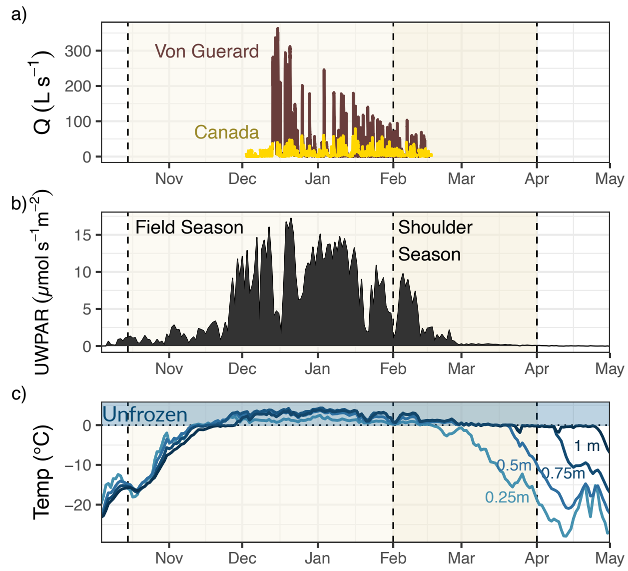

Figure 14. a) Streamflow in Von Guerard and Canada stream 2016-17. b) Underwater PAR and c) water temperature in the moat of Lake Fryxell 2018-19, highlighting the presence of liquid water in the moat (above 0°C) until May.

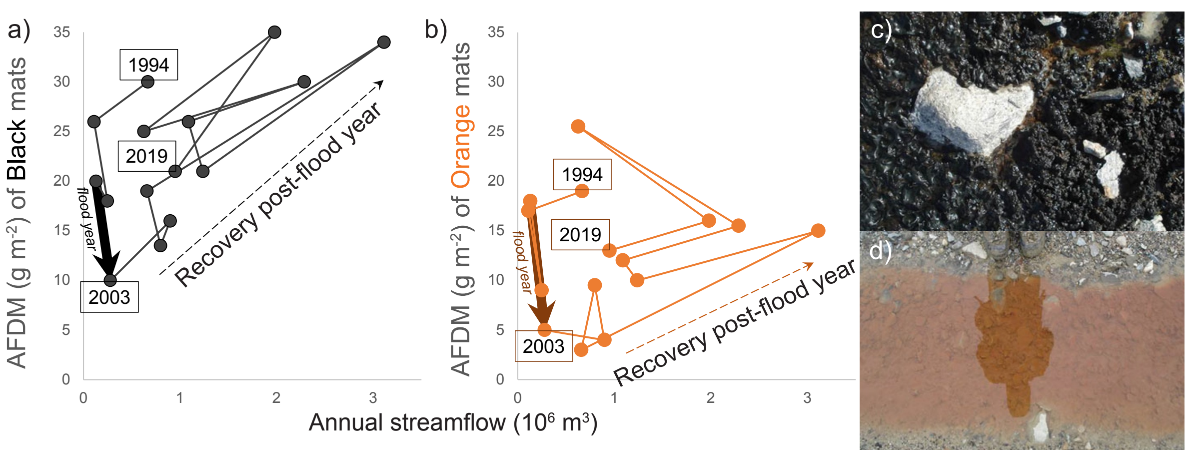

Figure 15. Relationship between annual streamflow and average stream benthic mat biomass, showing faster recovery in a) black (Nostoc) mats [c] relative to b) orange (Oscillatorian) mats [d] following the scouring of the 2002 flood. No data were collected during the flood. Images from Salvatore et al. 2020 and MCM core datasets.

3.2.3 Soil Lake Inundation Moat Experiment (SLIME) [Adams, Barrett, Doran, Hawes, Morgan-Kiss, Takacs-Vesbach, Mackey] - SLIME began in MCM5 to assess how lake-level change (i.e., changes in connectivity) influences the biogeophysical properties of the soil, aquatic, and benthic ecosystems. We established transects on the north and south sides of FRX and ELB, with two seasons at FRX (2017-18, 2019-20) but only one season at ELB (2018-19) due to Covid-19. We compared the lateral spatial distribution of bacterial and eukaryal communities (16S rRNA and 18S rRNA amplicon sequencing) and the abundance of microscopic fauna *Scottnema lindsayae*, *Eudorylaimus sp*, *Plectus frigophilus*, and tardigrades (*Acutuncus antarcticus*). In Year 3, we ran a pilot Moat-Mat-ICE

Connectivity experiment (MMIC) to test whether early season thaw of frozen mats mobilized organic and inorganic resources for the open water moat communities. SLIME revealed community differences between the moat and the limnetic regions of the lakes (Fig. 16), differences in biodiversity as a function of depth, and a threefold increase in thawing mat chl-a in the moats.

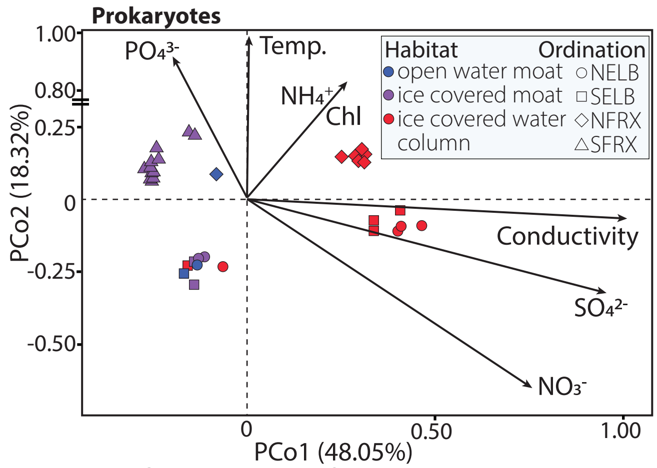

Figure 16. PCoA ordinations of bacterial communities in the moats and stratified water columns of Lake Fryxell and ELB.

Soil to shallow moat sampling will continue on an annual basis in MCM6 at both FRX and ELB, in addition to MMIC deployment in both lakes. We will conduct lateral surveys of water column communities during Years 2 and 5 (bbe FluoroProbe, 16S/18S rRNA gene sequencing at FRX, in addition to shore-based sampling of shallow mat in the Fryxell and ELB moat). We will also develop a shared sampling protocol with SMaLS (§3.1.4) to integrate future observations across landscape units. In the Year 4 extended season, we will 1) resample water column and shallow mat transects ahead of February shallow moat freezing, and 2) augment existing benthic and water column oxygen sensors with surface deployed dataloggers to monitor redox conditions over the protracted freeze-up (Fig.14).

Diving will occur in Years 1, 3 and 6 at FRX only, and will include resampling the sites described above under both the permanent ice and in the moat. Diving activities will be coordinated with the new DIM experiment (§3.3.4). SLIME mat transects will extend to below the ice cover in MCM6 to assess the long-term fate of nutrients fixed in moat mats as lake level rise submerges them. The shallowest under-ice extent of most MDV lakes features buoyant disruption of microbial mats (Fig. 17) due to dissolved gas supersaturation (Craig et al. 1992). Buoyant lift off mats can separate from the benthos, freeze into the base of the ice cover and, over the course of years, migrate to the ice surface. Once at the surface, mats are subject to aeolian transport that exports nutrients and viable microbial communities from the lake benthos to terrestrial environments (Parker et al. 1982, Wharton et al. 1983). We will assess 1) the processes driving gas supersaturation that are responsible for microbial mat lift off, and 2) the extent of benthic mat nutrients and biomass exported by lift off processes at FRX. We will develop a lift off index from mat morphology and style of mat/sediment layering in Year 1 and dive to map the spatial extent of buoyant disruption. Sampling will include lift off mat, residual sediment, and intact mat along the SLIME depth transect. Repeat drop camera surveys at transitional depths along the SLIME transect will be conducted in both early and late season to assess changes to lift off mat extent correlated to monitored lake characteristics including moat connectivity and melt water influx. A relative index of locally

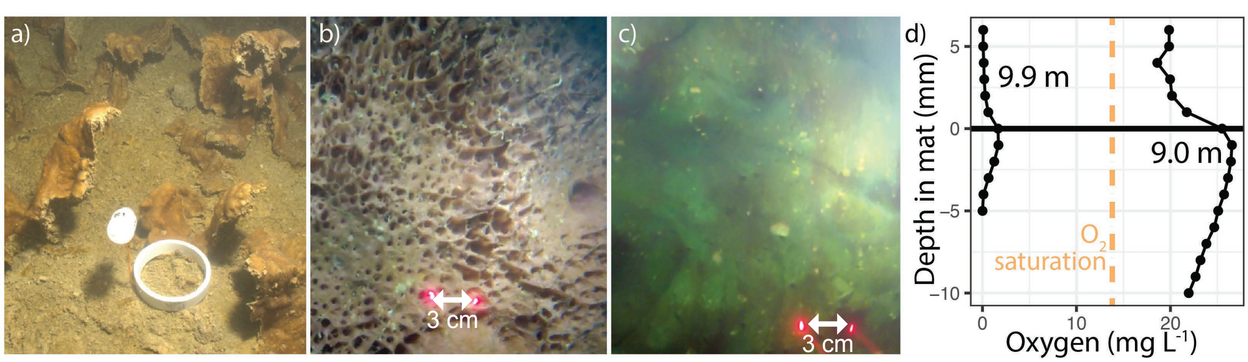

Figure 17. Benthic microbial mats and geochemical microenvironments in Lake Fryxell. a) Microbial mats at 5.6 m subject to liftoff to be investigated as part of SLIME (3.2.3). b) Benthic mats spanning the Lake Fryxell oxycline, including c) green biofilm dominated by *Phormidium pseudopriestleyi* at 9.9 m. d) Dissolved oxygen microelectrode data from benthic mats. Beneath the oxycline, the mat at 9.9 m creates an oxic environment (Sumner et al. 2015).

exported mat will be determined from paired upward-facing cameras capturing the abundance of liftoff mat on the underside of the ice cover. Correlation of liftoff mat observations with lake conditions will inform predictive models for future changes in mat lift off and estimating changes in mat nutrient contributions to other landscape units through the history of long-term observation in the region.

SLIME activities will test all hypotheses of MCM6. Both the benthic mats and water columns show evidence of shifts in key taxa laterally through the moats, and comparison of their responses to abiotic drivers provides a test for H1. Ecological legacy is impacted by variations in the size of the moat and the hydrological connectivity between the moats and the stratified water column, testing H2. Our assessment of lift off mat contribution to aeolian nutrient fluxes will provide a test for H3 in the carryover of nutrients among hydrologically isolated landscape units. The freeze-up and MMIC experiments will test H4.

3.2.4 Lake and Moat Integrated Connectivity Experiments (X-ICE) [Morgan-Kiss, Takacs-Vesbach] - The LICE and MICE experiments were designed to determine the effect of increased ecosystem connectivity on lake water productivity, as well as community composition and functioning. We incubated dialysis-bagged materials from selected landscape components in microcosms of surface and moat water from P-deficient ELB and N-deficient FRX to simulate increased material transport resulting from increased hydraulic connectivity. In ELB, primary and bacterial productivity increased in the microcosms receiving P treatments. In FRX, primary productivity was stimulated by the N treatments as expected, but bacteria were not, indicating that not all communities will respond similarly to increased connectivity. In MCM5, we conducted transplant-LICE and -MICE experiments (tLICE and tMICE) to investigate the impact of lake level rise on microbial community diversity and functioning. Dialysis-bagged communities from ELB and FRX were transplanted for 14 days to either the moat or deeper in the water column. We discovered that shallow communities exhibited both higher sensitivity and homogenization to the mimicked disturbances of increased connectivity (tMICE) or lake level rise (tLICE), while deeper communities were resistant to the transplants (Sherwell et al. *In review*).

In MCM6 Year 4, LICE will be conducted *in situ* using the ALPS-RAS sampler in combination with the PPS (to monitor native communities) and the ITP. The RAS sample bags will be preloaded with either nutrients or leachate. RAS and PPS will be programmed to collect after one week (to allow lake ice holes to refreeze) and samples will be collected at 2, 4, 6 and 8 weeks. Replicate RAS bags will be used for biogeochemical analyses (chlorophyll, DOC, IC and nutrients). MICE will be conducted concurrently in the open water moats in RAS bags, in addition to tLICE and tMICE (with additional timepoints). *These experiments will test the H1 prediction that the abundance and activity of sentinel taxa will change in response to simulated disturbance. We will test the H2 prediction that ecological legacy, e.g., the permanent chemoclines, confer higher resilience to environmental disturbance, while near-surface communities which experience the impacts of variations in lake surface properties will exhibit higher sensitivity to environmental disturbance.*

3.3 New Experiments.

3.3.1 Seasonal Soil Manipulation (SSM): Biotic responses to seasonal variation in soil temperature and water availability [Adams, Barrett, Salvatore] - Antarctic soil organisms, e.g., bacteria, archaea, and microscopic invertebrates, are essentially freeze-dried for all but 10-12 weeks each year. Even during the austral summer these organisms are typically subject to many freeze-thaw cycles (Knox et al. 2016). As a result, resident biota must survive extreme variations in water availability, temperature, and illumination over daily, seasonal, and interannual timescales. This experiment will examine the mechanisms of soil biotic community stability under natural and experimentally manipulated physical (temperature) and hydrological variation.

To assess mechanisms of biotic recovery following desiccation and rehydration, we will install an *in situ* open top warming chamber experiment (*sensu* Simmons et al. 2009), following 5 treatments from mid-November through mid-February (and longer into the austral autumn during our extended shoulder season). The 5 treatments will include open top chamber (+T), wetting the surface soil to field capacity (+W), open top chamber + wetting (TW), and an unmanipulated control. Open top chambers warm the soil by an average of 2.2°C in this region (Simmons et al. 2009). Chambers will be deployed in mid-

November at the beginning of the austral summer (**Obryk et al. 2020**) and remain in place through mid-February to assess the influence of warming on the frequency of freeze-thaw cycles and the composition and functioning of microbial communities. Wetting treatments will be administered as previously described (Simmons et al. 2009) twice during the season: once in mid-December, and once in mid-January to evaluate the influence of wetting during different times of the summer season when temperatures vary. By assessing soil organismal, population, and ecosystem responses to wetting treatments, we will identify potential differences in community composition and physiology associated with seasonal freeze-dry, thaw, and wet-up cycles (Table 3). Sentinel soil taxa will include *Scottnema*, *Eudorylaimus,* and cyanobacterial mats where present. Hyperspectral reflectance data will be acquired concurrent with sampling using an ASD FieldSpec4 spectroradiometer (350 - 2500 nm) to correlate key ecosystem characteristics with observed changes to the spectral properties of these ecosystems. These results will help to refine our spectral detection limits and our ability to estimate photosynthetic biomass in soils using remote sensing data (Salvatore et al. 2021a). Collectively, these data will allow us to quantify community resistance and resilience (Orwin and Wardle 2004, Griffiths and Philippot 2013, Todman et al. 2016). For example, we will compare control and +T plots to examine the resistance of soil communities and ecosystem processes to warming. Additionally, we will assess resistance as the total change in diversity, composition, and activity of a post-wetting community with respect to a pre-wetting and/or control community, and we will characterize resilience as the degree to which populations, community composition, and activity returns to a pre-wetting state using a quantitative index developed by Orwin and Wardle (2004), and recently applied to the drought-rewetting responses of soils exposed to different land-use history (Osburn et al. 2021). *This experiment addresses H1, H3, and H4 by defining population, community, and ecosystem responses to and recovery from changes in abiotic drivers.*

3.3.2 PSE (Permafrost Subsidence Experiment, formerly PDE) [Adams, Barrett, Diaz, Gooseff, Salvatore] - Winds deposit sediments, salts, and biologic materials in streams at high frequency and generally low magnitude, apart from seasonal foehn deposition events. As stream channels deepen and widen due to increased and prolonged warming and meltwater flow, permafrost subsidence is another important vector for material deposition to streams, connecting soils to aquatic systems. Permafrost subsidence events typically occur at low frequency but high magnitude. We anticipate these events to occur at a higher frequency in a warming future as subsurface ice becomes less stable. Streambank subsidence and degradation occurs most frequently in stream channels lined by massive buried ice deposits, which are common in the eastern extent of Taylor Valley (Fig. 18) and the southern extent of Garwood Valley, and are uniquely susceptible to thaw by directly exposing subsurface ice (Levy et al. 2013, 2018). When substantial channel erosion has occurred in the past, stream solute loads increased, potentially fertilizing downstream ecosystems (Fig. 18; Gooseff et al. 2016). However, no previous studies have tracked the influence of streambank subsidence from the onset of the event through time to understand the influence of high magnitude deposition events on stream biology and biogeochemistry.

In this experiment, we will identify one location for each of three streams in three basins with varying nutrient limitations (i.e., N-limitation in Fryxell Basin vs. P-limitation in Bonney Basin due to geochemical legacy effects) and varying benthic mat density during one summer. For each

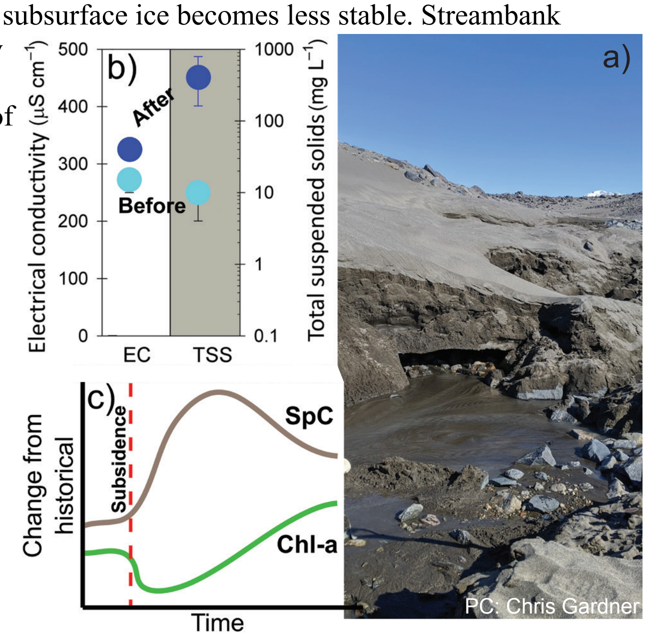

Figure 18. a) Naturally occurring permafrost subsidence. b) Crescent Stream conductivity and suspended load before and after flow through subsided permafrost, and c) prediction of SpC and chl-a response from PSE.

stream, nearby thawed soil/sediment ($0.5m^3$) will be transported into the stream channel to simulate increased landscape connectivity due to permafrost subsidence. Background geochemical, mat composition, and UAV-derived data will be collected for two seasons prior to manipulation and monitored for two seasons. UAV-derived topography and Terrestrial LiDAR Scanning (TLS) data (see *Letter of Collaboration - UNAVCO*) will be collected to document the channel form up- and down-stream, and benthic mat samples will be collected for AFDM, chl-a, diatom, and invertebrate analyses, and to estimate *in situ* primary productivity using pulse-amplitude modulation chl-a fluorescence at several locations up- and downstream of the sediment input. Synoptic water chemistry samples up- and downstream will be collected pre- and post-deposition (upstream as a reference for downstream changes). We expect that sediment will be transported and deposited downstream, modifying channel form, which we can monitor using both UAV- and satellite-based remote sensing. The fresh sediments will be leached and weathered upon immediate and prolonged contact with stream water, potentially enriching stream waters in weathering solutes and nitrate (similar to Gooseff et al. 2016) and algal mat, diatom, and invertebrate communities will change compared to conditions prior to the start of the experiment, dependent on nutrient storage in the stream channel and sediment redistribution. *This experiment, which is explicitly linked to the biology and water quality monitoring following disturbance of the PSE (§3.3.2), tests H2 and H4. Depending on mat abundance and drift sequence, we expect the impact of stream subsidence to vary in different streams, confirming the importance of physical and ecological legacy in H2. If changes in biological composition, chl-a, and nutrient abundance persist in the seasons following the streambank subsidence simulation (i.e., high magnitude disturbance), H4 will be supported.*

3.3.3 Varying Additions of Stream Inorganic Nutrients (VAIN) experiment [Gooseff, Zeglin, Diaz, Salvatore] - In MDV streams, like many streams globally, hyporheic zones and benthic autotrophic mats are locations of extensive nutrient uptake (Gooseff et al. 2003, McKnight et al. 2004, Cozzetto et al. 2013) and transformation (**Kohler et al. 2018**, Heindel et al. 2021). To learn this, we used short-duration stream nutrient additions; however, our newest findings lead to a question of how increased nutrient availability due to extended increases in total flow and shifts in hydrological hysteresis (Singley et al. 2017) will alter net stream nutrient retention. In other whole-stream nutrient enrichment experiments, including in the Kuparuk River, Alaska, the strongest biological responses emerged after long-duration change and in nutrient-poor systems (Peterson et al. 1993, Ardón et al. 2021). We propose a long-term fertilization experiment on Wales Stream to simulate increased mobilization of nitrates from hyporheic sediments, a predicted consequence of enhanced stream flow connectivity (**Singley et al. 2021)**.

Wales Stream has low total nutrient concentrations but is high in dissolved soluble reactive phosphorus (~50 µg-P/L) relative to dissolved inorganic N (~120 µg-N/L), with a stoichiometry of 3N:1P (Welch et al. 2010, Olund et al. 2018), and discharges to the ocean rather than a closed-basin lake (thus, the experiment will not impact MDV lake research). Nutrient enrichment will elevate NO_3 concentrations to a peak of 1.6 mg-N/L and SRP concentrations to a peak of 100 µg-P/L, to achieve a 16N:1P ratio. This is an optimal N:P demand ratio for basal autotrophs and heterotrophs in both aquatic and terrestrial ecosystems (Ho et al. 2003, Cleveland and Liptzin 2007), yet concentration targets still fall within the high end of the measured range in MDV streams, and are representative of concentrations in local soils and aeolian sediments (Bate et al. 2008, **Diaz et al. 2018**). The experiment design will assess the influence of nutrient fertilization frequency and magnitude on benthic mat biomass and community

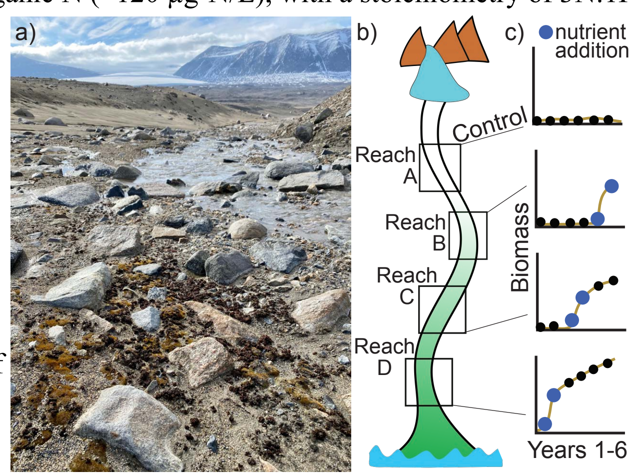

Figure 19. a) Wales stream and exposed mats. b) Set-up of VAIN, with experimental reaches (B-D). c) Hypothesized results from nutrient additions on biomass. Blue dots are years receiving nutrients, black dots are monitoring years.

dynamics, nutrient limitation, retention and transformation during seasonal flow. We will identify 4 reaches from upstream to downstream, A, B, C and D (Fig. 19). Reach A will always serve as an upstream control with no nutrients added. In Years 1 and 2 of the project, weekly pulse injections of N and P will be added in reach D; in Years 3 and 4, constant rate injections (for 8 hours) will be conducted on reach C once per week (sampling at the end of both C and D); and in Years 5 and 6, we will aim for a constant rate addition from at the top of reach B (sampling at the end of B, C, and D). Three times in each season (before treatment, middle and end of season) we will perform all standardized stream monitoring activities (§3.1.4), including geochemistry, biology, and remote sensing, with the addition of hyporheic geochemistry and stream dissolved oxygen (DO) to estimate metabolism, to determine how the integrated stream biogeochemistry changes through time in response to the fertilizations.

We predict that a biomass growth response in sentinel taxa (dominant autotroph relative abundance and biomass, as well as stream metabolism) over the six-year experiment will be greatest in reach D and decrease going upstream. Further, we expect nutrient retention and biomass to be positively correlated, and that benthic mat communities will decrease in diversity, with endemic taxa becoming more rare and cosmopolitan taxa more dominant. Finally, we predict that the retention of nutrients added with these three different patters will cause different levels of temporal carryover (local nutrient surplus, H3). *This experiment will test H1 by tracking population, community, and ecosystem responses concurrently; H3 will be tested through an evaluation of comparisons in responses to the variable frequency of fertilization; H4 will be tested through evaluation of processes during the extended season (§3.3.5).*

3.3.4 Decreased Irradiance on Mats (DIM) Experiment [Mackey, Doran, Hawes] - DIM will explore the response of benthic mats to changing irradiance that occurs through 1) annual variations in cloud cover (Acosta et al. 2020), ice cover transmissivity (e.g., snowfall, early melt and clouding of ice Howard-Williams et al. 1998, Myers et al. *In revision*) or water column attenuation (e.g., suspended sediment pulses or planktonic blooms), 2) multi-year variations in ice cover transmissivity due to the delivery and long-term residence of aeolian sediment (e.g., Rivera-Hernandez et al. 2019), or 3) decadal or unidirectional change such as lake level rise (e.g., Hawes et al. 2011). Primary productivity in benthic microbial mats is largely carried out by cyanobacteria with extreme shade adaptation (Moorhead et al. 2005, Quesada et al. 2008) and potential reductions in PAR would decrease photosynthetic rates in these benthic ecosystems as PAR fluxes are below photosystem saturation (Hawes and Schwarz 1999, 2001).

The DIM experiment will involve deployment of ~1 m^2 benthic shades installed 10-20 cm above the mat-water interface across the FRX oxycline. Each shade will comprise two light reduction treatments: one opaque and one with a slat lattice for a uniform 50% PAR transmission. Shades will be placed in triplicate at three depths along a benthic transect across the lake oxycline, encompassing a range of redox microenvironments and different patterns of N cycling (cf. Jungblut et al. 2016, Dillon et al. 2020a, 2020b). Experimental monitoring will involve comparison of benthic mats subsampled in replicate from unshaded, partially, and fully shaded treatments to assess the implications of local metabolic activity on ecosystem functioning under different ambient oxygen levels. *In situ* pore water chemistry will be measured via microelectrode profiling (pH, O_2, H_2S, redox) and MiniSippers (Chapin and Todd 2012) will sample pore water nutrients. The effect of treatments on mat community composition will be measured by 16S, 18S rRNA gene amplicon sequencing and infauna microinvertebrate surveys, with the net influence on benthic accumulation measured by organic carbon and carbonate accumulation. DIM installation and control sampling will take place in MCM6 Year 1 in logistical coordination with an existing 1-year shading experiment in FRX that focuses on the transition polar winter darkness to summer photosynthesis within the benthic mats (NSF award 1937748, Mackey co-PI). In Year 3, the control and one shade structure at each depth will be subsampled and have the shade removed, representing two years of decreased PAR. The remaining two shade structures will be left in place. In Year 6 the control and one additional shade structure will also be uncovered and subsampled, representing 5 years of decreased PAR. At this point, the shade structure uncovered in Year 3 will be resampled to assess resilience of the community following the preceding PAR reduction. The final shade structure will be left in place pending support for assessment of ecosystem resistance to longer-term shading. *This experiment will directly* *assess H1 with prediction of trophic-level variability in how PAR disruption would influence ecosystem functioning, including sentinel mat taxa (§3.1.8). H2 will be assessed through the relationships of 1) resistance of mat community composition and nutrient cycling to PAR disruption and legacy lake redox and 2) influence of legacy photosynthetic biomass on disruption. H3 will be tested in the relative resilience of taxa with different metabolic needs and trophic levels with PAR perturbations.*

3.3.5 Shoulder Season Activities [All PIs] - Each year, the MDV ecosystem undergoes extreme seasonal shifts in environmental conditions. The transition to winter means a reduction in light and liquid surface water. Endemic organisms have the physiology to thrive through these dramatic changes, as soil and stream communities become active again each summer, and lake communities shift between photosynthetic and non-photosynthetic processes (Lizotte et al. 1996, **Patriarche et al. 2021**). However, the environmental cues that organisms respond to in preparation for polar night, the extent of inter-seasonal shifts in community structure and functioning, and whether the polar day-night transition can be classified as an annual ecosystem disturbance, are all still unclear. To address these gaps, we plan to collect our established data streams into April (i.e., capturing the polar night transition) in MCM6 Year 4 (2027). *This extended season serves as a natural experiment, specifically testing **H4** by evaluating how local and landscape-wide disturbances influence recovery from the polar night.* The following are activities we propose during this extended season:

Soils: Monitor occurrence of late season shifts in soil moisture through remote sensing and extend SSM (§3.3.1) into the shoulder season. Collect high resolution shallow soil profiles peak summer (mid-January) and in April to quantify changes in salts, nutrients, water content, and invertebrate abundance. This will test the hypothesis that nutrients and water are stored in the shallow subsurface and that nematodes move vertically in the soil column in response to physical seasonal change. Evaluating the vertical movement of nematodes in reference soils and at sites that have been disturbed (i.e., nutrients or water added in the past, (§3.2.1) provides a direct test of H4.

Streams: Install conductivity and soil moisture probes along a long-term reference stream channel bed, and Wales Stream (§3.3.3) before winter dry-down, collect high resolution shallow stream channel sediment profile for geochemistry and biology, and monitor biomass changes using manual methods and remote sensing (§3.1.4). This comparison will improve our understanding of the nutrient and C storage in stream channels and shifts in community structure and productivity during and following drying and freezing, and of the resources available to fuel stream activity the following season.

Lakes: Conduct limno runs and SLIME transects (§3.2.3) to measure physical and chemical parameters, primary productivity, bacterial activity, and community composition. This sampling, coupled with decades of summer data, will determine compositional and metabolism shifts throughout the water column and moats, and determine whether legacy nutrients and/or contemporary productivity are sustaining photosynthetic organisms during periods of no light. We will also extend *in situ* manipulation experiments (LICE, tLICE §3.2.4) into the polar night transition to directly test the impact of disturbances such as nutrient flux and limitation on late season processes. Last, we will employ lab-controlled experiments to provide physiological data on responses of sentinel phytoplankton taxa to mimicked polar night (§3.1.8).

3.4 Modeling [Dugan, Gooseff, Barrett, Salvatore].

We will develop and test models to 1) provide a quantitative framework for the hypotheses of this proposal and 2) leverage the long-term datasets collected in MCM1-5 to predict future change in the MDV ecosystem. PIs have expertise in mechanistic lake modeling (Dugan), hydrological and biogeochemical modeling (Gooseff), community modeling (Barrett), spectral mixture analysis and radiative transfer modeling (Salvatore) and, collectively, integrated modeling activities with individual experiments and long-term monitoring sites.

Lake models will be integrated with the deployment of high-frequency sensors and sampling of microbial communities in the Lake Bonney Narrows, which separates WLB from ELB. Time-series analysis of high frequency data will be used to build a mechanistic model of how external and internal drivers (wind and subglacial input) control the frequency, magnitude, and duration of deep-water spill-over events from the hypolimnion of WLB into ELB that shape habitat and potentially community dynamics. High-frequency data will also be used to update our understanding of how connectivity shapes the stability of lake thermal and density profiles, how the lake physical habitat may have changed in the last 50 years (since Shirtcliff 1964), and whether a change in climate drivers and glacial input could change lake and community stability in the future (testing H1 and H3).

MDV streams provide test cases for spatio-temporal dynamics of connectivity as they are locations of hydrologic connectivity and receive aeolian deposition of minerals, nutrients, and organic matter (§3.1.6). Streams also serve as locations of within system ecological connectivity (**Kohler et al. 2018**), with a predictable downstream direction. We will develop a new quantitative mechanistic model that integrates the temporal and spatial dynamics of ecological connectivity (i.e., downstream transport of organisms, cycled nutrients and organic matter) and physical connectivity to predict nutrient and carbon cycling in streams (Fig. 20). This model will be informed by aeolian deposition (§3.1.6), hydrology and stream chemistry (§3.1.4), benthic mat dynamics (§3.1.4, SMaLS), and direct measurements of synoptic stream and hyporheic water chemistry, stream particulate organic matter fluxes (Cullis et al. 2014), and whole stream metabolism, in Von Guerard Stream. MCM monitoring activities are well-established at this site, so building and calibrating the model here will add significant value to our long-term datasets. We will apply the model to two other streams with extensive records - Green Creek (short stream carpeted with benthic mats) and Lost Seal Stream (short-moderate length stream with a sand bed and few mats) to explore different scenarios of stream structure and functioning. Ultimately, we will be able test all four working hypotheses using this model, by simulating disturbances (high flows, scouring, etc.) and changes in the magnitude, frequency, and duration of both hydrological and aeolian connectivity in/to streams.

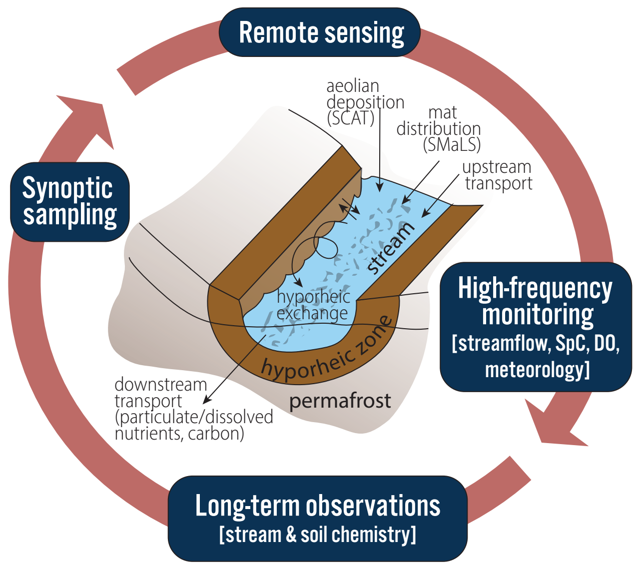

Figure 20. Stream modeling of water and solute transport informed by remote sensing, synoptic sampling, high-frequency monitoring, and long-term observations.

We will continue to refine understanding of terrestrial biotic communities (invertebrate, biocrust communities and microbial mats) in the MDVs using a combination of remote sensing, our long-term datasets, and species distribution modeling efforts (e.g., **Andriuzzi et al. 2018a**, 2018b, Power et al. 2020, **Sokol et al. 2020**). We will use remote sensing methods (Salvatore et al. 2020, 2021a) to characterize the spatial and temporal variability of hydrological and physical variables that influence species distribution, i.e., mineralogy, surface salts, and water content. These data will be incorporated into the MCM Data Catalog to inform statistical species distribution and mechanistic models of community assembly (e.g., Poage et al. 2008, **Sokol et al. 2020**) and to generate habitat suitability maps throughout the MDV over a range of past, present, and future climatic conditions. This modeling activity will allow us to test how changes in the frequency, magnitude, and duration of physical drivers influence terrestrial communities (relevant to H1, H2, and H3) and predict composition of sentinel taxa under different climate scenarios. We will validate these next generation models of habitat suitability using ongoing surveys of soil communities (e.g., Thompson et al. 2019, 2020, 2021).

4. RELATED RESEARCH PROJECTS

MCM LTER is the longest running and most comprehensive research project in terrestrial Antarctica, and we lead international efforts that guide responsible stewardship of the MDVs (Priscu and Howkins 2016). Our approach to conducting research and broader impacts requires us to achieve all our objectives solely from LTER grant resources. Thus, our PI group is intentionally small compared to other LTER sites.

However, we amplify our capacity to inform our hypotheses and advance scientific discovery through additional NSF awards and collaborations with a wide array of other US and international research projects in the MDVs. For example, our recent FSML award (NSF 2114156) provides significant upgrades to datalogger and telemetry infrastructure associated with our long-term meteorological, soil, stream, and lake monitoring networks as well as new sensors to support our hypotheses regarding year-round impacts on biological processes. Other NSF supported projects include regionalized patterns and processes of biotic diversity and distribution (NSF 1341736), remote sensing of NPP and habitat suitability (NSF 1744785, NSF 2046260), stream Fe weathering and biogeochemistry (NSF 1841228), genetics of microbial interactions (NSF 1937546), photosynthesis of sentinel psychrophiles (DOE SC0019138), paleolake deposits (NASA 80NSSC22K070) and their chronology (NSF 1946326), and onset of lake oxygenic photosynthesis (NSF 1937748). Our work also leverages joint MDV projects with scientists supported by the national programs of New Zealand, UK, Ireland, Spain, Italy, Korea and South Africa.

5. BROADER IMPACTS

Just as our hypotheses for MCM6 are centered around the frequency, duration, and magnitude of change and connectivity, so are our proposed broader impacts and how they relate to educational and outreach activities. Inspired by our collaboration with PAL on the Polar Literacy Project (NSF 1906897), we plan to create and review principles for communicating our research though "Antarctic Core Ideas" (Fig. 21). These are principles about Antarctic science that we believe all people should understand: they will provide a framework for all our outreach and education work. These principles will anchor the breadth of outreach and education activities by requiring that all activities address one or more of the core concepts, in a similar way that our experiments are connected to the central hypotheses. We will test the efficiency of our broader impacts by evaluating how effective we were in communicating the Antarctic Core Ideas and how that translates to broadening participation.

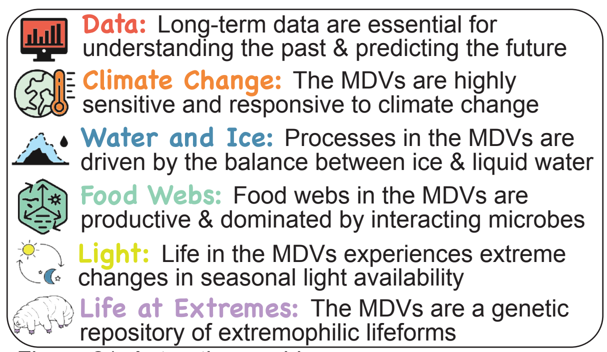

Figure 21. Antarctic core ideas.

5.1 K-12 Outreach.

MCM PIs have a long history of engaging with K-12 students and have engaged with over 4,000 students each MCM funding cycle. We will expand participation with K-12 teachers and develop new activities, in addition to our Antarctic Core Ideas, that will significantly extend the broader impacts of this project. In addition to established classroom visits both virtually while in Antarctica and in person in our respective communities, we will develop new collaborations among scientists, artists and teachers with the goals of expanding our schoolyard program to include professional development of K-12 teachers and diversifying our strategies for communicating our science.

Woven into all our efforts is a commitment to reaching historically underrepresented groups in science, technology, engineering, and mathematics (STEM). We will do this through cultivating connections with community partners and schools, and by thoughtful use of formative and summative evaluation to ensure we're accomplishing our goals. We are particularly interested in whether our programming is promoting STEM learning activation in young participants: are we increasing participants' fascination with science, changing values about the natural world, belief in their own competency as scientists, and their comfort with learning and trying new things? To assess effectiveness, we will use survey instruments developed by organizations such as the Learning Activation Lab and the STEM Learning and Research Center (STELAR), as well as non-survey methodologies.

5.2 Undergraduates and Early Career Scientists.

5.2.1 Summer REU program - We have learned through our previous efforts at the SACNAS Annual Meeting that Antarctica alone is not enough to attract significant numbers of students historically underrepresented in STEM to polar research. Rather, we will highlight the societal benefit of our environmental research (as recommended in Carter et al. 2021) in recruiting efforts and throughout a ten-week REU program to be held in Year 2. We will recruit six REU students through our institutions' undergraduate diversity programs (e.g., McNair Scholars at UNM and KSU; NAU's Louis Stokes Alliance for Minority Participation; MU's Bridges Program; CU's BOLD Center) and nationally at the annual SACNAS meeting. These long duration outreach activities can have a profound impact for supporting future generations of polar scientists. Students will be recruited to the ten-week program based on their desire to pursue a graduate degree and interest in exploring a career in polar research. Students will select an MCM LTER host lab where they will conduct an independent research project, mentored by an MCM PI and supported by a MCM graduate student near-peer mentor.

REUs and their near-peer mentors will participate in a cohort-wide weekly virtual environmental data training program led by Takacs-Vesbach and Brown (a certified Carpentries instructor). For the first five weeks, these meetings will include a two-hour Data Carpentry (Teal et al. 2015) session, which will teach students best practices in data organization and reproducible workflows and include basic training in R programming. We will host a weekly journal club co-led by alternating MCM PIs and a one-hour professional development session (e.g., applying to graduate school, selecting a mentor). In week six, the REUs and their mentors will convene at UNM for a four-day intensive workshop to test basic ecological theory on resilience using time series modeling and the application of critical slowing down (Carpenter et al. 2011) as a metric to predict tipping points. These data dives will be taught by PIs Dugan, Salvatore, Takacs-Vesbach, Morgan-Kiss, and Zeglin. In the final weeks, REUs will continue to meet in preparation for the annual MCM Summer Meeting in August, where they will each give an oral presentation.

Takacs-Vesbach is the Director of the UNM MARC/U-RISE program and has run a similar program for five students each summer since 2016 (89% of those students are currently in PhD programs). The effectiveness of the MCM REU program will be evaluated by UNM's Institution for Social Research using many of the same approaches as the UNM MARC/U-RISE program. Based on the evaluator's report, we will modify our REU program and hold a second REU program in year 5 of the grant.

5.2.2 AntRECCS - We are developing an annual semester-long course and research program to engage six community college students in the MCM LTER called AntRECCS (Antarctic Research Experience for Community College Students) led by PIs Diaz and Gooseff, with support from all PIs. Logistical and financial support will be provided by MCM, INSTAAR, and CU Boulder. The program builds off the established Research Experience for Community College Students (RECCS), which is a long-standing summer internship program for Earth sciences at CU Boulder. The community college (CC) students from partner institutions in Colorado will take a class co-taught by Diaz and Gooseff about the history of Antarctica and research best practices, and work on a research project with MCM6 PIs. The course will end with a symposium involving CU and the partner colleges. CC students will gain hands-on experience with Antarctic research and develop/strengthen science communication skills. AntRECCS will engage 30 CC students during MCM6 (5 per year) and 30 middle school classes through targeted classroom visits. The cohorts will have the opportunity to connect with each other and the broader MCM community at the annual MCM Summer Meeting. The CC population is more ethnically and racially diverse than that of CU Boulder, Boulder County, and the physical sciences as a whole (Malcom 2012, Bernard and Cooperdock 2018). By recruiting and supporting CC students, we will help to diversify Earth science and ecology early career researchers through this high frequency and long duration program.

5.3 Arts Integration into Science Communication.

Inspired by our success collaborating with both PolarTrec and the Antarctic Artists and Writers Program, we plan to focus our Schoolyard Programming in MCM6 on the theme of using art to engage students in a meaningful way with our science. This model is motivated by recent research supporting the idea that academic achievement and self-efficacy are improved through integrating arts and science education

(Best et al. 2019). We plan to use the Data Jam model in informal, out-of-school science learning settings. Data Jams challenge students to make creative projects (such as songs, physical models, stories, infographics, and games) that convey complex ecological data to nonscientists. First, we will bring one artist or one teacher (alternating) into the field in years 2-5. Teachers and artists will be recruited via a competitive application process: we will prioritize teachers connected to schools supporting a majority historically underrepresented student body, and artists who are committed to science communication and or education. Projects will be connected to one or more of our Antarctic Core Ideas (Fig. 21). Both artists and teachers will be expected to connect with and participate in the lab activities of their host PI prior to their deployment. Post-deployment, they will work closely with CU Science Discovery staff to co-design curricular activities for formal and informal settings (see *Letter of Collaboration*). They will participate in a professional development workshop for science and art teachers, with the goal of supporting teachers in developing data literacy skills, polar literacy, and using art to bring creativity, engagement, and cultural awareness to the process. We will use PolarTREC resources and training modules as a basis for recruiting, selecting, and preparing our teachers and artist collaborators.

We plan to support an annual Art Exchange collaboration between artists and project graduate students, inspired by the Flow Project at the University of Wisconsin campuses. Artists and graduate students will partner to collaborate on projects creatively explaining a students' data and science story to a public audience – a sort of Data Jam for artists. We anticipate displaying images associated with these projects in an exhibition, making the curated datasets available on our outreach website and social media accounts, and using them in our teaching and outreach work. We anticipate that this new, long duration initiative will engage a high magnitude of students and community members.

6. CONCLUDING REMARKS

Dependent upon glaciers and underlain by frozen ground, the MDV ecosystem is poised for significant change in the face of climate warming. Through our past research, we have focused on the influences of landscape and ecological legacies, contemporary connectivity, and how these processes influence the structure and functioning of this ecosystem. Ecosystem linkages driven by climate produce a cascade of tightly coupled events that ultimately lead to the biological production, diversity, and biogeochemical dynamics that occur in the MDVs. Like all ecosystems, the MDVs encompass dynamic interactions among biotic and abiotic components: after 30 years of research on this particular system, we are poised to define the timescales at which the mechanisms underpinning ecosystem resilience operate. Thus, the science proposed in MCM6 is a natural evolution of the ideas from MCM1-5. We have refined our approach to linking past legacies and contemporary processes, pushing our concept of connectivity beyond a binary definition to encompass the temporal duration, frequency, and magnitude of connectivity. This approach will help to advance our understanding of an ecosystem that thrives at the boundary conditions for life on Earth, thus may be particularly sensitive to small changes the frequency, duration, or magnitude of ecological connectivity. Significant changes to our PI group provide us with new energy, new perspectives, and new expertise. This group will forge discovery in the MDVs while mentoring an increasingly diverse group of students, postdocs, and technicians, with a goal of changing the future face of polar science and ecology. Based on a legacy of foundational scientific data that are well-curated and easily discoverable, we have developed exciting new outreach programs, trained new generations of scientists, and now propose transformative research for extending our scientific and educational contributions even more effectively into the future.

MCM6 Data Management Plan

1 Introduction. The McMurdo Dry Valleys LTER (MCM) is a data-intensive research endeavor that conducts a broad range of monitoring and experimental studies that span temporal and spatial scales. Accordingly, Information Management (IM) is an integral component of our research program. The primary goals of MCM IM are to 1) facilitate and advance scientific research in the McMurdo Dry Valleys (MDVs) region of Antarctica by ensuring the timely availability and long-term preservation of high-quality, well-documented, and publicly accessible data and metadata, 2) keep current on new technologies, trends, and best practices in research data management, 3) provide education and training to promote sound practices around the data life cycle, and 4) actively engage with other data management professionals and researchers in the LTER Network and other relevant research-focused communities.

The MCM IM System (IMS) integrates all components of the DataONE data life cycle (Michener and Jones 2012) to host the most comprehensive scientific database for the MDVs. In addition, the IMS encompasses several other aspects critical to the overall functioning of our research program (e.g., cyberinfrastructure, education and training, broader community engagement). We manage and curate all MCM-funded data, including data generated by other federally funded research projects that leverage MCM resources, and adhere to data standards and access policies outlined by the US LTER Network, the US National Science Foundation (NSF) Office of Polar Programs (OPP), and the US Antarctic Program (USAP). All MCM datasets are documented with rich human and machine-readable metadata, made freely available under the Creative Commons Attribution 4.0 International License (CC BY 4.0), and archived in a timely manner in the Environmental Data Initiative repository (EDI), enabling discovery through DataONE. To ensure MCM data are discoverable through the Antarctic Master Directory (AMD) and fulfill data sharing obligations under the Antarctic Treaty, metadata records associated with MCM grants are registered with the US Antarctic Program Data Center (USAP-DC) with data links pointing to the MCM Data Catalog (hosted by the MCM IMS) and to the master inventory of MCM datasets in EDI.

2 Resources

2.1 Personnel. The MCM IMS is based at the University of New Mexico (UNM), where it has been under the continuous oversight of PI Takacs-Vesbach since the start of MCM4, ensuring ongoing continuity in our IMS infrastructure and personnel. Renée Brown became our full-time Information Manager at the start of MCM5 after working at SEV LTER for 15.5 years as a system administrator and lead instrumentation technician. Brown is a skilled technical professional and experienced research ecologist whose responsibilities include all aspects of the MCM IMS (e.g., data management, system administration, website, databases, telemetry, training). During the 2018-19 field season, she worked alongside MCM team members in the MDVs for two months, gaining invaluable first-hand experience. Brown is joined at UNM by a strong community of data management professionals, including three other LTER Information Managers, half of the EDI team, and UNM Library's Research Data Services (RDS) team. Brown and Takacs-Vesbach attend all biweekly virtual MCM Leadership Team and biannual in-person PI and Summer Science meetings, and Takacs-Vesbach serves as the IM representative on the MCM Executive Committee. This ensures all members of the MCM Leadership Team (see *Project Management Plan*) are informed about new research developments, field logistics plans, and dataset status. All members of the MCM team play important roles in the MCM IMS, working closely with the IM team throughout all stages of the data life cycle from proposal development and experimental design to data archiving and publication. In between, all team members generate and validate datasets associated with long-term *core* and shorter-term *non-core* research projects. When *core* datasets are not submitted on time, Brown, Takacs-Vesbach, and Lead-PI Gooseff work closely with the responsible PI(s) to address issues and facilitate submission. In addition, MCM REUs periodically train with Brown to develop new IM tools. The MCM Project Manager and the graduate student representative to the LTER Network also assist the IM team in keeping the personnel database and listservs updated. In MCM6, we will expand team involvement in the design and implementation of our IMS with a formal IM committee that will empower students, postdocs, technicians, and other PIs to become more engaged with the data life cycle.

2.2 Cyberinfrastructure

2.2.1 Servers, databases, and storage. The MCM IMS backbone consists of robust physical server infrastructure housed in a dedicated rack in a secured-entry, climate-controlled, and generator-backed server room at UNM that is shared with EDI. In MCM5, we upgraded aging equipment to common architectures to increase efficiency and ease future IMS transitions. One Dell PowerEdge R330 server, running Ubuntu Linux 20.04.3 LTS, hosts the MCM website and associated databases (including the MCM Data Catalog), while a second, running Microsoft Windows Server 2019, hosts the Campbell Scientific LoggerNet software, used by the MCM Telemetry Network, as well as the Aquatic Informatics AQUARIUS Time-Series platform and associated PostgreSQL database used for processing MCM hydrological data. Servers are configured with 2-4 TB RAID 5 storage arrays to provide built-in backup redundancy and long-term storage capacity. Updates occur regularly to ensure security and performance. Server and database access occurs over secure authentication protocols (e.g., SSH, VPN) and along with website editing privileges and IM documentation, is limited to the IM team to preserve data integrity and protect sensitive information. Centralized relational databases have been integral to our IMS since MCM1, and we recently migrated from proprietary infrastructure (Oracle) to an open-source and widely respected database solution (PostgreSQL 14.2). In addition to consolidating services into a unified database solution, this was also a critical early step in our multiphase plan to implement a new metadata database and website (see *Section 6*). Several cloud-based services also complement our physical server infrastructure. Dropbox and Google Drive provide secure shared access to data and other information across our institutions. Website usage is tracked using Google Analytics. GitHub and GitBook provide collaborative version-controlled repositories for IM tools and documentation, and Asana is used to manage IM workflows. Crashplan provides continuous server backups that are tested periodically to ensure recovery. Additionally, all MCM data and metadata are archived on EDI servers to ensure long-term preservation and provide the recommended tertiary level backup of our data.

2.2.2 Telemetry. The MCM Telemetry Network was established in the MDVs during the 2010-11 field season to enable year-around acquisition of environmental sensor data, facilitate pre-season planning and within-season troubleshooting, reduce costly helicopter-supported site visits, and serve as an additional backup of *core* meteorological and hydrological datasets. In a hybrid network topology consisting of Iridium satellite modems (part of NSF's polar Iridium contract) and serial radios, data are retrieved using a master Iridium hub that was relocated from CU Boulder to UNM in MCM5 to consolidate telemetry services and improve network performance. Provisional data are made publicly available in near real-time on the MCM website via interactive *R* Shiny dashboards designed by a former MCM REU student mentored by Brown. In addition, MCM maintains two web cameras in the MDVs that are connected to a separate telemetry network managed by UNAVCO (see *Letter of Collaboration*). Recognizing the need to enhance and expand environmental monitoring in the MDVs, particularly in the context of MCM6 hypotheses regarding year-round impacts on biological processes, Brown co-wrote an NSF infrastructure improvement proposal with PIs Doran and Gooseff that was successfully funded in 2021 (DBI-2114156). As part of this project, Brown will work with an MCM REU student to make necessary improvements to our online data visualization dashboards that will not only resolve recent connectivity issues, but also incorporate our camera imagery and other new data streams.

3 Data Life Cycle

3.1 Planning. There are several distinguishing features involved in conducting research at MCM that present unique data acquisition constraints. These include the high degree of coordination required in planning each field season (inevitably requiring on-the-fly changes due to highly unpredictable and variable weather conditions during the field season), the limited number of personnel who can deploy each year, limited physical access to MDV research sites, and the relatively short field season. Moreover, extreme Antarctic conditions (e.g., sub-zero temperatures, katabatic winds, six months of darkness) create additional challenges and opportunities for automated data collection using sensor networks.

3.2 Collection. MCM data are generated primarily from physical samples collected during the field season or from in-situ sensor networks that run seasonally (e.g., stream gages) or year-round (e.g., meteorological stations, lake monitoring platforms). *Core* datasets are associated with long-term, ongoing monitoring projects or experiments, and overseen by one or more responsible PIs, while *non-core* datasets reflect opportunistic and other shorter-term studies, including student-led research. Along with sample collection, establishment and maintenance of field research sites occurs via small teams comprised of graduate students, seasonal technicians, and PIs. Most field-collected samples are subject to laboratory analyses, either performed in MDV field camps and McMurdo Station, or shipped to our US-based institutions for further processing. Provisional telemetered data are provided in near real-time on our website, and all sensor data are manually downloaded during the field season for further processing prior to archival. Occasionally, *core* research sites (especially stream gages) have become inundated by rapid lake level rise (Fountain et al. 2014) and relocated or decommissioned. *Core* datasets may also be discontinued over time to balance available resources and accommodate new research directions.

3.3 Assurance. Researchers are responsible for validating their respective datasets, which include sensor generated data, prior to submission to Brown. She then performs additional quality assurance and quality control (QA/QC) checks that include assessing dataset structure and metadata completeness, and makes additional improvements and corrections as needed. Tabular data are then imported into the master MCM relational database where they are subject to additional built-in and programmatic constraints (e.g., proper cell formatting, consistent naming conventions, no duplicate rows). Finally, as part of the EDI archival process, MCM data packages must pass a variety of congruency checks, which include assessments of FAIR Data Principles (i.e., *F*indable, *A*ccessible, *I*nteroperable, and *R*eusable; Wilkinson et al. 2016).

3.4 Description. All MCM datasets are richly described by human- and machine-readable metadata using the Drupal Ecological Information Management System (DEIMS). As a comprehensive database-backed (via MySQL) metadata solution, DEIMS supports easy entry, editing, and display of human-readable metadata from which Ecological Metadata Language (EML), a widely used metadata standard supported by EDI and DataONE, is automatically served, enabling efficient one-click upload of MCM data packages into EDI. DEIMS also incorporates several other features, including persistent digital object identifiers (DOIs) that link directly to MCM data packages in EDI, the LTER Controlled Vocabulary service (Porter 2010), and the LTER Unit Dictionary service (Kortz 2009). The process for submitting new datasets begins with the metadata form (adapted from EDI). Researcher(s) meet virtually with Brown to work through the form together, providing the opportunity to address questions and concerns while also increasing transparency regarding the data life cycle. Metadata associated with existing *core* datasets are reviewed at least once annually by the IM team and associated PIs, and updates are ongoing.

3.5 Preservation. The MCM data use and intellectual rights agreement was rewritten in MCM5 to better align with open science principles and the LTER Network Data Access Policy (LTER Science Council 2017). Accordingly, we strive to have all *core* data archived in EDI within one year of collection, and all *non-core* data archived prior to the publication of associated manuscripts, barring instrument failures, personnel turnover, the pandemic, and other unanticipated obstacles that may arise. MCM data are released under an open licensing scheme (CC BY 4.0) allowing anyone to freely reuse, redistribute, transform, or build upon our data so long as they are properly cited (Brown 2021). Data citations are anticipated to provide an important metric regarding the reuse of our data as good data citation practices become more widely adopted by the broader research community.

3.6 Discovery. MCM data are discoverable through the MCM Data Catalog, hosted by the MCM website, as well as several other online portals, including data repositories (e.g., EDI, USAP-DC) and aggregator services (e.g., DataONE, AMD). The MCM website provides a centralized public-facing portal for our research program that includes relevant background information, personnel directories, a fully searchable up-to-date bibliography, current and past proposals, research highlights, links to education and outreach efforts, and more. In MCM4, the website underwent a substantial redesign with the adoption of DEIMS, a custom full-featured web content management platform that has provided a powerful IM solution for numerous research sites in the LTER Network and beyond (Gries et al. 2010, San Gil 2013, Wohner et al. 2019). The MCM Data Catalog provides a highly curated, fully searchable, and centralized online database through which visitors can easily access all MCM data. Datasets are broadly organized within MCM research areas (e.g., meteorology, glaciers, streams, lakes, soils, integrative processes), relevant subcategories (e.g., biology, chemistry, locations), and *core* data status. Faceted search tools enable further discovery by keywords (e.g., subject, personnel, location) and LTER core areas (i.e., disturbance patterns, inorganic nutrients, organic matter, primary production, and population studies). Interactive dashboards of telemetered sensor data are also provided through a locally hosted *R* Shiny application server. Like most LTER sites, the vast majority of MCM data are archived in platform agnostic tabular formats in EDI. However, generation of non-tabular data (e.g., imagery, models) has been increasing, along with interest in improving discovery of other non-traditional data (e.g., field notebooks, protocols, code), as well as data better suited for archival in specialized repositories. For example, nucleotide sequence data are required to be archived in an International Nucleotide Sequence Database Collaboration repository, such as GenBank (Field et al. 2008, Yilmaz et al. 2011). While an extensive listing of genomic datasets is provided through the MCM Data Catalog, we have begun linking genomic datasets associated with new MCM data packages following recently published best practices (Gries et al. 2021) to improve discovery of MCM genomic data through EDI and DataONE. In MCM6, we plan to further incorporate these best practices in archiving new non-tabular datasets, such as remote sensing and modeling products.

3.7 Integration and Analysis. Our extensive use of relational databases ensures that datasets generated across the project use common, harmonized data structures (e.g., variable names, units). Built-in database constraints and use of controlled vocabularies further support efficient integration and analysis of MCM data. To assist with the analysis of time series data, derived data packages (e.g., daily means) are provided for *core* meteorological and hydrological data. In MCM6, we also intend to contribute to the still-in-development ClimHydroDB data harmonization effort to support synthesis activities across the LTER Network. MCM researchers are responsible for the analysis of their respective datasets. Internal syntheses across the project are fostered by regularly sharing new results with other members of the MCM team during our biweekly virtual and biannual in-person meetings, as well as at our annual graduate student research showcase held virtually each spring. While many of our research products are published as individual datasets, we frequently synthesize our results in highly collaborative papers developed within the project (e.g., **Gooseff et al. 2017a**), across the LTER Network (e.g., Bowman et al. 2016, Iwaniec et al. 2021), and internationally (e.g., Dragone et al. 2021).

4 IM Education and Training. Brown regularly provides updates and formal presentations on IM policies, procedures, and best practices during biweekly virtual MCM Leadership Team meetings and biannual in-person PI and Summer Science meetings. Given the highly distributed nature of the MCM project, in-person meetings provide important opportunities for researchers and the IM team to connect face-to-face. In addition, Brown held an informal data jam at our 2019 Summer Science Meeting for students, postdocs, and technicians. Informal assessments have proven the effectiveness of IM training efforts through improvements in both *core* and *non-core* data submissions, a marked increase in *non-core* data submissions prior to publication, and better data citation practices. Since 2012 (pre-MCM), Brown has regularly organized and co-taught a popular hands-on short course on environmental sensor network fundamentals, which has trained four MCM graduate students to date including incoming MCM6 PI Dugan. Since joining MCM, Brown has continued this workshop, which now includes a data management component. In 2019, she partnered with NTL LTER to co-teach a spin-off workshop, inspired by her original course, focused on in-situ aquatic sensing using open-source technologies. While in-person training has largely been on hold due to the ongoing pandemic, we intend to resume these opportunities in MCM6 for graduate students, postdocs, and technicians, potentially in collaboration with other LTER sites, as well as for our REU students (see *Project Description* §5.2.2). Early in MCM5, Brown became engaged with the EPSCoR-funded New Mexico Carpentries program through her collaboration with the UNM Library's RDS team. Since 2017, she has served as a helper, instructor, and organizer in several in-person and virtual Software and Data Carpentries workshops. Brown became a certified Carpentries instructor in 2020, enabling her to independently organize and lead Carpentries workshops for the MCM team and will be an integral member in our new REU training efforts.

5. LTER Network and Broader Community Engagement. The MCM IM team maintains an ongoing commitment to active participation and leadership within the LTER Network and beyond. Brown regularly attends monthly virtual and annual in-person LTER IM Committee meetings, where she routinely organizes sessions promoting IM knowledge exchange. She also participates in several IM working groups, ranging from the development of best practices around non-tabular data to advancing data harmonization and synthesis efforts across the Network. In 2018, Brown was elected to the IM Executive Committee, and in 2021, as the IM representative on the LTER Executive Board (through 2024). Since 2020, she also serves as the IM representative on the LTER Diversity, Equity, and Inclusion Committee. Beyond LTER, Brown has served as an invited co-chair of the Federation of Earth Science Information Partners (ESIP) EnviroSensing Cluster (2017-21), a former LTER working group she co-founded in 2012, and was a member of the National Ecological Observatory Network's Soil Sensor Technical Working Group (2018-21). She has presented in and convened numerous sessions around research data management at ESIP, Scientific Committee on Antarctic Research (SCAR), and related meetings. Brown also represented MCM at the 2021 NSF Antarctic Subsea Cable Workshop that focused on efforts to bring broadband connectivity to the McMurdo region.

6 Future Initiatives. In MCM6, we will maintain our ongoing commitment to achieving the fundamental goals of MCM IM (see *Section 1*), improve the discoverability of MCM genomic data in EDI, and incorporate new remote sensing and modeling data products. In addition, notable improvements to our IMS will include the implementation of a new metadata database and subsequent overhaul of our website.

6.1 Metadata Database. The highly customized nature of DEIMS, combined with stalled development, has made it difficult to incorporate new features and technologies, such as more recent versions of Drupal and EML. After a thorough evaluation of metadata storage and EML generation approaches across the LTER Network, we plan to adopt the LTER Core Metabase as our new metadata solution in MCM6. This collaboratively supported open-source solution stores human-readable metadata in a PostgreSQL relational database (aligning with our goal towards a unified database solution), from which EML can be generated using a variety of *R* packages (Gastil-Buhl et al. 2019, Kui and O'Brien 2018, O'Brien and Gastil-Buhl 2013). Embedded integration of the LTER Controlled Vocabulary and Unit Dictionary services, combined with the power of relational database constraints, will continue to improve our data integration and analysis efforts. Additionally, this solution will enable us to adopt and implement newer EML features and perform bulk metadata updates of all MCM data packages. **Timeline:** *Complete migration of metadata associated with* core *datasets by the end of year three. Metadata associated with existing* non-core *and decommissioned* core *datasets will be ongoing as time and resources allow.*

6.2 Website Overhaul. DEIMS has provided an effective comprehensive solution for MCM IMS over the past decade, supporting metadata and data management needs as well as our bibliographic and personnel databases. Thus, migration out of DEIMS involves a multi-step process. Many open-source, community-supported solutions have become available since the development of DEIMS that we intend to leverage to simplify website management in MCM6. We plan to use the LTER Core Metabase to integrate our personnel database with our metadata and Zotero to host our bibliography. While we are still in the process of evaluating website platforms, we are particularly attracted to the numerous advantages provided by static website solutions (e.g., Jekyll), which are blazing fast, secure, simple to maintain, and can harness the power of APIs to include interactive features like the MCM Data Catalog (via EDI) and bibliography (via Zotero). **Timeline:** *Complete by the end of MCM6.*

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