Recharge from glacial meltwater is critical for alpine springs and their microbiomes

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Abstract

The importance of glacier meltwater as a source of mountain-block recharge remains poorly quantified, yet it may be essential to the integrity of alpine aquatic ecosystems by maintaining baseflow in streams and perennial flow in springs. We test the hypothesis that meltwater from alpine glaciers is a critical source of recharge for mountain groundwater systems using traditional stable isotopic source-identification techniques combined with a novel application of microbial DNA. We find that not only is alpine glacier meltwater a critical source of water for many springs, but that alpine springs primarily supported by glacial meltwater contain microbial taxa that are unique from springs primarily supported by seasonal recharge. Thus, recharge from glacial meltwater is vital in maintaining flow in alpine springs and it supports their distinct microbiomes.

1. Introduction

Mountain-block recharge (MBR) occurs when rain, snowmelt, or possibly meltwater from glacial ice, infiltrates and enters the saturated media beneath the water table in the high elevations of a mountainous watershed. There are considerable uncertainties regarding the partitioning of meltwater released from alpine glaciers, especially how much, if any, contributes to MBR. Traditionally, meltwater from alpine glaciers is assumed to leave catchments by (a) surface runoff as streams, (b) infiltrating and flowing through moraine sediment or alluvium, and/or (c) recharging shallow alluvial aquifers (figure 1). The bedrock of glaciated mountain blocks is commonly treated as an impermeable barrier due to glacial polishing, thereby limiting infiltration and eventual recharge into the mountain groundwater system. However, there is anecdotal evidence that alpine catchments can receive groundwater recharge specifically from glacial melt [1–3]. The magnitude of recharge from glacial meltwater and its role in mountain groundwater processes remain poorly quantified. This problem is exacerbated by geographical and geological variability in recharge processes.

We contend that glacial meltwater can infiltrate and recharge both shallow aquifers occurring within glacial moraines and sediments as well as deep mountain-block aquifers hosted within bedrock. Thus, the water balance of a glaciated alpine catchment should include recharge to both shallow and deep aquifers (figure 1). Alpine glaciers form in diverse lithologies around the world and the intrinsic controls on groundwater flow within these lithologies can be highly variable (i.e. different types of rocks have different porosities and permeabilities). In addition, the processes that create mountain ranges (orogenic processes) affect the porosity of the mountain block. Bedrock may have primary porosity, or porosity present when the rock was formed, although some crystalline rocks such as plutonic and metamorphic rocks may have very low primary porosity. Tectonic processes can create fault and fracture networks called secondary porosity, or porosity formed after the rock was formed. Secondary porosity in alpine catchments also forms due to processes such...
as glacial isostatic rebound and basal water pressure. Both of these processes can therefore facilitate groundwater recharge and enhance deep circulation both within the mountain-block and beyond to adjacent valleys [4]. Therefore, the geologic and tectonic history of a mountain system must be considered when investigating the contribution of glacial melt to groundwater recharge in these mountainous systems. To address this knowledge gap, we specifically investigate the presence and significance of MBR from glacial meltwater in dissimilar lithologies (figure S1 (available online at stacks.iop.org/ERL/16/064012/mmedia)).

Stable isotopic analyses of deep, regional aquifers and ice-sheet/groundwater models indicate substantial groundwater recharge occurred from melting continental ice sheets during the Pleistocene [5–7]. Seasonal snowmelt has supported MBR in mountainous regions around the globe since the Last Glacial Maximum (LGM). Over longer timescales, snowpack is transformed into glacial ice only to be released back to the active hydrological systems during glacial retreat. If and to what extent melting mountain glaciers contribute to MBR in modern alpine catchments remains unknown. Despite the importance of the world’s water towers [8], it is extremely difficult to decouple the magnitude of recharge from alpine glacier meltwater from that of seasonal snowpack in mountain groundwater systems. For example, timing of seasonal snowmelt and increased seismicity were correlated on a glaciated volcano in the Cascade Range, U.S. [9]. This observation suggested seismicity was caused by groundwater recharge, but seismicity alone cannot distinguish between the recharge of annual snowpack and glacial meltwater. Other studies in the Cascade Range have shown that stable isotopic values of water ($\delta^2$H, $\delta^{18}$O) from some low-elevation springs require a high-elevation recharge source, suggesting that recharge is sourced from snow or perhaps glacial melt [10, 11].

Stable isotopes ($\delta^{18}$O and $\delta^2$H) have been used extensively to identify the sources of recharge to aquifers in mountainous watersheds where rain and snow are the only potential sources of recharge [12–15]. While glacial ice is effectively composed of compounded seasonal snowpack over the past several hundred years, glacier ice and modern-day snow are commonly isotopically distinct since there have not been substantial isotopic excursions in precipitation over that same time interval. In fact, on a local meteoric water line (LMWL) plot, the isotopic composition of glacier ice falls in line with seasonal snow and rain (but is isotopically lighter than snow), thereby making it difficult to partition the meltwater using traditional isotopic separation techniques. Because alpine glacier ecosystems host extremophile microbial communities that can be isolated and unique to specific alpine and coastal polar regions [16–22], we propose that DNA sequence information endemic to glacier microbes can be used as an additional indicator of recharge from glacial melt.

Figure 1. Conceptual diagram of major hydrological components in a glaciated alpine catchment. Shallow or local flowpaths are denoted by small, short arrows while intermediate length flowpaths are shown with longer arrows. Groundwater can flow via primary porosity (pore spaces between grains), as well as through secondary porosity (fractures). The porosity of a mountain groundwater system varies with lithology and tectonic history.
Providing estimates on how much meltwater contributes to recharge and how much MBR from glacial meltwater supports perennial flow in springs and baseflow in alpine streams will significantly improve our understanding of the spatial and temporal extent of ecological responses to glacial retreat [16–19, 23]. A recent study specifically highlights that the ecohydrogeology of springs and their riparian habitats hold not only immense biodiversity and socio-cultural significance, but also enhances our hydrogeological understanding of groundwater systems [24]. As such, the incorporation of DNA as an environmental tracer for aquatic macroinvertebrates and fish populations is increasing in popularity due to cost-effectiveness and increases in availability. Similar applications using DNA to assess the microbial assemblages have been applied to track rainfall-recharge on Mount Fuji [25, 26], but this approach has not been used as a tracer of glacier meltwater. Given the likelihood that many alpine glaciers will disappear within this century, it is imperative that we quantify the role of meltwater from alpine glaciers on mountain groundwater systems and the significance of their aquatic ecosystems.

2. Methods

2.1. Overview
In this study, we combine a Bayesian isotopic end-member mixing model with 16S rRNA amplicon sequencing to identify the magnitude of recharge from glacial meltwater in alpine springs and streams. We target rapidly retreating glaciers in Mount Hood National Forest (MH) of Oregon, USA and Glacier National Park (GNP) of Montana, USA (figures 2 and S1, supplementary dataset S1: table 2). These sites vary by bedrock lithology and meteorological conditions, thus allowing us to examine the role of bedrock and seasonal precipitation on MBR. We analyzed samples of glacier ice, snow, rain, stream, and spring water collected in MH and GNP from 2016 to 2019.

2.2. Site description
MH is a stratovolcano in the Cascades with underlying geology consisting of andesitic lavas with deposits from pyroclastic and lahar flows from late-glacial through the Holocene [10, 27]. Very high discharge springs in the area (upwards of 283 l s⁻¹ at one location), in combination with published isotope and water chemistry data, reveal that springs cannot be topographically defined bywatersheds, as they appear to be discharging water from a larger area encompassing recharge at higher elevation area [10, 28, 29]. The glaciers on MH have decreased in area by approximately 34% since the beginning of the 20th century, but the sizeable glaciers still present, and easy access to them, makes this an ideal study site [30]. GNP, Montana is located in the Livingston and Lewis Range containing Mesoproterozoic and sedimentary formations with Cretaceous and sedimentary formations with Cretaceous shales underlying them [31]. The laminated, low porosity formations are highly fractured and some contain dolomitic units which allow fracture flow, as well as flow along contacts and through karstic conduit systems. GNP represents a severe case of retreat, as there are only 26 of 150 named glaciers in GNP remaining and model projections predict they will disappear between 2030 and 2080 [32–34].

2.3. Spring and stream water collection
Springs samples collected for this study were selected to achieve a broad spatial distribution with respect to elevation, geologic setting, and aspect. Most spring locations were identified by finding labelled spring locations on published topographic maps, while other locations were identified by satellite imagery, suggestions from National Forest Service and National Park Service scientists and staff, as well as previously published work [10, 11]. Samples of water for stable isotopic analyses were collected during the following sampling campaigns: July 2016, October 2016, July–August 2017, July–August 2018, and September 2019. While spring samples were preferred for this study, stream samples were collected when the region containing the spring source could not be physically accessed due to rugged terrain, safety protocols, or physical limits of the team regarding carrying weight. Differentiation of springs vs streams is noted in supplementary dataset S1: table 2. Water samples from springs and streams were collected using a 3D printed, portable peristaltic pump and Masterflex silicon tubing. The tubing was placed in the spring emergence and a filter was attached to the opposite end (see Microbial DNA methods section below for more information on filtering apparatus). Care was taken to ensure spring source areas were not disturbed. Stream samples were collected by placing the tubing into the stream. Water was pumped directly into new Nalgene bottles which were field rinsed three times with the water being collected at each site. Water samples were collected in 250 ml, 500 ml, or 1 l Nalgene bottles and the lids were sealed with electrical tape. Samples were stored in our vehicle in coolers at roughly 12 °C–15 °C while completing the fieldwork. Samples were stored in the coolers from a few days up to 3 weeks, depending on when samples were collected during the sampling campaigns. Once returned to the lab, the water samples were kept refrigerated until the time of analysis.

Water was pipetted from the Nalgene bottles to 2 ml glass autosampler vials with plastic screw caps for isotopic analysis. Maximum storage time from the time of collection in the field to pipetting and analysis was up to 2 months. Decontamination protocol for MH water sampling included using a 91% ethanol (C₂H₅OH) solution to decontaminate...
Figure 2. Sampling locations categorized by sample type for MH (A)–(E) and GNP (F)–(H) and surrounding regions. Inset boxes are denoted by corresponding border color. Spring and stream samples are shown in white, and those having SSU rRNA sequencing data are shown with an ‘X’. See supplementary dataset S1: table 2 for sample location details.

2.4. Isotopic analysis

The ice in MH and GNP is estimated to have been deposited a maximum of 7000 years ago, but peaked in the mid-1800s during the Little Ice Age [30, 35–37]. Therefore, the ice remaining today is likely a few hundred years old and is isotopically distinct from modern day precipitation. The isotopic end-member values for this study were selected based on representative samples of collected glacial ice, snowpack, and rain for MH and glacial ice and snowpack for GNP, as we assume these are the predominant sources of recharge in each respective region (table 1).

Analysis of $\delta^{18}O$ and $\delta^2H$ were measured by the Purdue Stable Isotope Laboratory (PSI Lab) using a Los Gatos Research, Inc. Triple Water Vapor Isotope Analyzer (model: 911-0034). The reported precision including correction for water vapor dependence, memory, and drift is 0.2‰ for $\delta^{18}O$ and 2.0‰ for $\delta^2H$. The values reported are relative to the Vienna Standard Mean Ocean Water–Standard Light Antarctic Precipitation scale (VSMOW-SLAP). The data is presented in figure 3.
Table 1. Isotopic end-member values and number of samples (n). End-member values are represented by the mean of the data. Site is abbreviated as MH for Mount Hood National Forest or GNP for Glacier National Park.

| End-member | $\delta^{18}O$ (%) | $\sigma$ $\delta^{18}O$ | $\delta^{2}H$ (%) | $\sigma$ $\delta^{2}H$ | n |
|------------|-------------------|----------------|------------------|-----------------|---|
| MH         |                   |                 |                  |                 |   |
| Glacier ice | -14.15            | 0.36            | -103.18          | 4.29            | 3 |
| Snow       | -11.72            | 0.75            | -84.60           | 5.61            | 14|
| Rain       | -10.18            | 0.85            | -70.06           | 7.51            | 6 |
| GNP        |                   |                 |                  |                 |   |
| Glacier ice | -18.07            | 0.31            | -135.47          | 1.51            | 2 |
| Snow       | -15.37            | 0.70            | -116.89          | 7.06            | 8 |

2.5. Isotopic end member mixing model

The Bayesian Monte Carlo mixing model is a modified version of Arendt et al [39]. The strategy used is that samples of a prior probability density function (PDF) are either accepted or rejected in proportion to the likelihood of the data, and yield samples of a posterior PDF [39]. The form of Bayes’ theorem applied here is:

$$ p(f) \propto p'(f \mid l) L(o \mid f \mid l) \tag{1} $$

where $f_i$ are the fractional contributions, $o_i$ are the isotopic measurements, $l_i$ are the isotopic composition of the end-member components, $p(f)$ and $p'(f \mid l)$ are the prior and posterior PDFs respectively, and $L(o \mid f \mid l)$ is the likelihood function [39]. The priors of the fractional contributions are that of ice ($f_i$), snow ($f_s$), and rain ($f_r$) and use the assumption that these are the three possible sources of recharge. The prior PDF includes variance in the isotopic compositions because each end member is not represented by a single value. Additionally, we assumed that the end member compositions are normally distributed with standard deviation given by the measurement uncertainties. We also assume the uncertainties of the isotopic compositions of the end members are uncorrelated. For MH, the fractional constraint is represented by $f_i + f_s = 1$, while GNP is represented by $f_i + f_r = 1$. We assume that all combinations between 0 and 1 are equally likely that the uncertainties of the measured isotopic compositions are purely Gaussian and uncorrelated. Therefore, the data likelihood function is given by

$$ L \propto \exp \left( \frac{(\delta^{18}O - \delta^{18}O_o)^2}{2\sigma_{\delta^{18}O}} \right) \times \exp \left( \frac{(\delta^{2}H - \delta^{2}H_o)^2}{2\sigma_{\delta^{2}H}} \right) \tag{2} $$

where $\delta^{18}O_o$ and $\delta^{18}O_o$ are the predicted and observed measurements of $\delta^{18}O$, $\delta^{2}H_o$ and $\delta^{2}H_o$ are the predicted and observed measurements of $\delta^{2}H$, and $\sigma_{\delta^{18}O}$ and $\sigma_{\delta^{2}H}$ are the measurement uncertainties. The predicted isotopic values are calculated using a standard linear mixing model. A Monte Carlo sampling scheme is used where random samples of the prior are retained as samples of the posterior proportional to the likelihood based on the misfit between predictions and data [39]. The subsequent rejection or acceptance of the prior samples retained are relative to the most likely model. Samples of the prior are accepted if the likelihood is 1, accepted half of the time if likelihood is 0.5, and never accepted if the likelihood is 0 [39]. Roughly 100 prior samples were tested and as described above, the posterior samples retained were in proportion to the relative likelihood of the associated prediction. The output of the model provides the best estimates of the fractional contribution of each end member along with mean values and standard deviations of the posteriors. Samples that were found to have a fractional contribution greater than 0.60 of glacial ice were identified as being ‘glacially influenced’ or having substantial glacial recharge. This threshold value was selected after performing sensitivity tests on the model using the endmembers from this study. Section 2.6 outlines additional information regarding this sensitivity study. If a location was sampled multiple times, the isotopic value providing the maximum glacial influence was selected for that site to locate all spring/stream locations containing potential glacial melt contribution.

2.6. Isotopic model sensitivity study

Isotopic samples that had a fractional contribution from glacial ice of 60% or greater are defined as being glacially influenced. This threshold was determined by creating known fractions of ice, snow, and rain representing 50 theoretical spring samples. The fractional values were created using a random uniform distribution. The known fractional values of ice, snow, and rain were used to calculate the theoretical $\delta^{18}O$ and $\delta^{2}H$ values that were then run through the models. To see how well the models estimate the fractional contribution from glacial ice, the same endmember zones that were used in the analysis of the field-collected MH and GNP samples were also used for this sensitivity study.

The fraction of ice, snow, and rain were calculated for the theoretical MH samples. Likewise, the fraction of ice and snow were calculated the theoretical GNP samples. The standard deviation of the of the fractional values predicted by the model act as upper and lower bounds for the estimated fraction
Figure 3. Dual stable isotopic plot for MH (A) and GNP (B). The $\delta^{18}O$ and $\delta^2H$ values for precipitation trend predictably, falling along the global meteoric water line (GMWL) \[^{38}\]. The endmember zones used in the mixing model are shown in color. Values for the end members are shown in table 1 and isotopic values of the data are listed in supplementary dataset S1: table 2. Spring and stream samples are shown by black outlined circles, and those having SSU rRNA sequencing data are shown with an 'X'. Samples shaded in teal represent the samples that receive significant contribution from glacial ice according to the isotopic model. Error bars shown include water vapor dependence, memory, drift, as well as normalization of the samples to the VSMOW/SLAP scale. The GMWL is presented on both plots. A local meteoric water line (LMWL) was created for MH by fitting a linear regression through the precipitation (snow and rain) samples collected. A previously published LMWL for MH is also shown \[^{10}\]. The LMWL for GNP was created by applying a linear regression through previously published USGS data. Additional information regarding the GNP LMWL can be found in figure S2 and supplementary dataset S1: table 1.

contributions. Therefore, if the model is performing well, the assigned theoretical fractional values should fall within the upper and lower standard deviation bounds.

For this study, we were particularly interested in identifying, with certainty, which springs receive a large fraction of water from glacial ice. This is accomplished by assigning a threshold value for the fraction from ice, where springs having greater than this value receive a significant amount of flow from glacial ice. This means that additional springs in this study may also be composed partly of glacial melt, but at lower fractions, because although this model works it does have limitations. The threshold value chosen was 60% and was determined by assessing above sensitivity study’s results. Specifically this was done by identifying at what percentage the model could no longer estimate the fractional contribution of glacial ice in
the sample. In laymen’s terms, this means that below 60% ice, the assigned fractional value of ice was no longer consistently falling within the standard deviation bounds provided by the model. Often, above the 60% threshold the model underestimated the contribution, providing a conservative estimation. The sensitivity study is presented in the supplementary material.

2.7. Microbial DNA collection, extraction, and rRNA gene sequencing
Filtering was completed by either filtering the water in a sterilized, negative pressure hood using a vacuum apparatus and (12 h, 450 °C) GF/F sterilized filters (0.22 μm and 0.45 μm pore size; Sterlitech Corporation) after sample return to the lab, or filtered in the field using individually sealed, gamma irradiated, sterile, polyethersulfone pressure filter unit membranes (0.22 μm pore size; Sterivex). Between 250 ml and 3 l of water was filtered per site. Snow and ice samples were first melted and then filtered. Field-filtered samples were filled with RNALater and capped on both ends for preservation and stored at −20 °C after transport until nucleic acid extraction. These filters were stored in falcon tubes for transport purposes. Prior to extraction, the samples were washed with 18.2 MΩ cm−1 water to remove RNALater. DNA was extracted from filters using a Qiagen PowerLyzer PowerSoil kit according to the manufacturer’s instructions. An unused filter or 18.2 MΩ cm−1 water served as a negative control during each round of DNA extractions. No DNA was detected in any negative controls. Concentration and quality of DNA were assessed using a Qubit fluorimeter and 1% agarose gels, respectively. We interpreted high quality DNA as high molecular weight whereas low quality/degraded DNA was apparent as low molecular weight bands. Using relative proportions, we assigned a quality score to each DNA sample between high, medium, and degraded. This quality check will serve as a relatively quick and inexpensive test to check for the viability of DNA across sample types and residence times. Samples that did not yield detectable amounts of DNA (using the Qubit™ dsDNA HS Assay Kit) were not sent for sequencing. The detection limit for the Qubit™ dsDNA HS Assay Kit is around 10 pg μl−1. Despite not detecting DNA in our negative controls, these were submitted for sequencing. All negative controls failed to pass quality control (performed by the University of Minnesota Genomics Center (UMGC)) and no sequence information was obtained.

To evaluate microbial community composition, the V4 region of the 16S rRNA gene was sequenced at the UMGC using a dual-indexing approach modified from the Earth Microbiome Project protocols [40] and MiSeq Illumina 2 × 300 bp chemistry generating 25 000–30 000 reads/sample. Post sequence processing was performed with mothur [41, 42]. Community composition was visualized using R software packages. Specific package citations are listed in the supplementary information.

3. Results

3.1. Isotopic model results
The stable isotopic data for this study plots along the Global Meteoric Water Line (GMWL) and the end member ranges provide appropriate separation for a mixing model (table 1, figures 3 and S2, supplementary dataset S1; table 2). The isotopic analysis shows that numerous springs in MH and GNP are supported by recharge from glacial melt. In MH, 88 total samples including end members and spring/stream samples were collected over the course of the sampling campaigns, with 38 of these being individual spring/stream locations. Our isotopic analyses show that 26% of the locations contained more than 60% glacial melt contribution to total flow. In GNP, 44 total samples were collected with 22 being individual spring/stream locations. Of these, 54% contained more than 60% glacial melt. The results from MH and GNP provide support for our hypothesis that alpine glacier meltwater is an important source of recharge for alpine springs. These results are plotted graphically in figure S3, spatially in figures S4 and S5, and in table format in supplementary dataset S1: table 4.

The importance of glacial-melt recharge is not confined to springs located close to glaciers. For instance, MH springs supported by glacial-melt recharge were more than 10 km away from glacial ice. In contrast, in GNP, low permeability rock and structural and stratigraphic controls on groundwater flow restrict the influence of glacial-melt recharge to springs closer to glacial ice. In both locations, groundwater does not always follow topographic watershed divides and the south-facing glaciers are retreating faster than the northern and eastern slopes. Therefore, in MH, high-elevation springs in the northeast discharge higher proportions of glacial melt than those towards the southwest. Springs discharging groundwater from deep, regional flowpaths (regional at the mountain-block scale) supported by glacial-melt recharge emerge at the lower flanks of the volcano perhaps due to large-scale faults in the area (figure S4, towards both the northeast and southwest corners of inset B). The glacially influenced springs in the southwest corner are also warm springs and previous studies inferred that they are discharging recharge from high-elevation snow or glacial melt [10, 11]. Our isotopic results indicate that they are discharging a large proportion of recharge from glacial melt (figure S5).

The groundwater systems in GNP are very different than MH; they are dominated by local-to-intermediate flow at higher elevations due to the stratigraphic and structural controls which effectively
Figure 4. The log relative abundance of the top 20 most abundant operational taxonomic units (OTUs) present in MH and GNP springs and streams. Bar plots of the top 20 most abundant OTUs are colored based on the genus-level taxonomy. The top two quadrants show the taxa of springs identified as having snow/rain as their dominant recharge, while the bottom two quadrants show the taxa of springs identified as having glacial melt as their dominant recharge. Bars with a log relative abundances of zero or below have extremely low abundances.

truncates groundwater circulation depths. Most of the remaining glaciers in GNP are within north-northeast facing cirques and their surficial catchment drainages are oriented mostly towards the northeast. However, we observe high-discharge glacially influenced springs emerging on the opposite side of the topographic divide containing glaciers and their runoff (figure S1 part B). We attribute this discharge to groundwater flow along fractures, geologic contacts, and/or karst-like conduits through the mountain bedrock to the spring emergence following the attitude of the geologic units which dip to the southwest (figures S1 and S5). The glacially influenced springs originating at low elevations are likely receiving the glacial water via fracture flow over much longer timescales.

Please note that all samples were collected in July, August, and September of 2017, 2018, and 2019 at various elevations and aspects in MH and GNP in an attempt to include representative, well-mixed examples of winter precipitation from snow and rain, and summer precipitation in the form of rain. We recognize that there can be considerable variability in isotopic values both between storms and seasonally; however, published data in MH and GNP either do not exist or are very spatially and temporally sparse prohibiting spatiotemporal comparison. MH has no published modern precipitation data to our knowledge, with the exception of a three samples of snow without latitude or longitude data [10] and three snow samples with only δ¹⁸O being reported [43]. GNP only has single snowpack values taken in March during the early 2000s that support our end-member range for snow (see supplementary dataset: table 1). Rain samples were collected in MH over two days in September 2019 to provide an end-member estimation. Glacial ice in both locations was sampled where accessible at the toe of the glacier and represents an estimate of the oldest glacial ice and therefore offers the most isotopically light values, distinct from modern precipitation. Additionally, for this study, the majority of samples collected are from springs. However, in cases where either the spring (or catchment containing the spring) could not be accessed, or there was interest in the isotopic composition of the stream, a sample from the stream was collected.

3.2. Microbial results

We used the results from the isotopic model to categorize the springs as ‘largely glacially recharged’ and ‘largely snow/rain recharged’ and coupled these data to DNA sequencing of 16S rRNA genes (in bacteria and archaea) in 60 of the 132 total samples of spring/stream, rain, snow, and glacier ice samples (figures 2 and 3). Springs with more than 60% glacial melt contribution contain taxa that are unique compared to springs receiving recharge mainly from modern day snowmelt and rain, proving additional support for our hypothesis (figure 4; figures S6–S10, and supplementary dataset S1: tables S5–S7). Many taxa are abundant in both springs supported by recharge from glacial melt and in snow or rain recharged springs, but the distinguishing characteristic is the taxa unique to glacially recharged springs or snow/rain recharged springs. Furthermore, springs supported by recharge from glacial meltwater in MH
have microbial communities overall that are distinct from glacial melt-recharged springs in GNP. Collectively, our Bayesian isotopic end-member mixing model with 16S rRNA amplicon sequencing demonstrate that (a) MBR from alpine glacier meltwater is appreciable, and (b) springs that are supported primarily by recharge from glacier meltwater contain microbial communities unique to the glacially recharged grouping.

4. Discussion and conclusions

We have shown that the proportion of MBR from alpine-glacial meltwater is comparable to recharge from modern day precipitation in some springs, with approximately 26% of the sites in MH and 54% in GNP being primarily supported by glacial meltwater. In stark contrast to previous studies, our data show that a substantial proportion of glacial meltwater may remain in the catchment (i.e. in the mountain groundwater system). This finding cannot be overemphasized since this recharge ultimately supports perennial flow from mountain springs and baseflow in mountain streams, processes that are vital to the future integrity of alpine ecosystems. Although alpine glaciers have been present since the LGM, this finding also suggests that these springs are dependent upon a temporary source of recharge. The permanence (and vulnerability) of these springs depends on the volume of water stored in the mountain aquifer and the response time of the aquifers to future changes in recharge. Many springs, like those discussed in this study, may disappear or experience decreased discharge in the future. The time lag between the disappearance of the alpine glacier and the desiccation of the alpine spring is controlled by the groundwater response time. A recent study showed that groundwater recharge predictions in the Columbia River Plateau Aquifer remain uncertain in future projections [44]. If there is a modest increase in recharge this could yield an increase in total recharge in this region, however there is an equal possibility that future increases in precipitation might not be able to overcome future increases in evapotranspiration (as vegetation encroaches on high elevations) and decreases in snow water equivalent [44]. However, in regional-scale predictions like these, it is even more difficult to make predictions for MBR because prior knowledge of groundwater routing in mountain systems is limited and further complicated in glaciated regions [44].

Springs receiving more than 60% of recharge from glacial melt contain unique microbes compared to springs receiving most recharge from modern day snow and rain. This could be due to transport of microbes originating in glacial ice. Glacial melt-supported spring water may also have different geochemical and/or thermal conditions which supports distinct microbial communities compared to streams supported by snow/rain. Regardless, there are taxa present in the glacier ice that are not present in the snow/rain recharge, and likewise there are snow/rain taxa that do not appear in the glacial recharge (figures S6–S10 and supplementary dataset S1: tables 5–7). This work highlights the microbial similarities and differences in glacial melt recharged springs between two geographically and geologically distinct study sites. When cross comparing the most abundant Phyla of the two field locations, MH and GNP have commonalities such as Proteobacteria and Bacteroidetes, but each also contain taxa unique to the locale. Distinctions in biogeography are likely a combination of bedrock lithology, precipitation, and season which constrain the distribution and composition of snow and ice microbiota [45–48].

There are several limitations to our study: we lack widespread samples of glacial ice due to the hazards associated with sampling near retreating alpine glaciers (e.g. rock fall/slides, glacial dam failure, etc), our data set is limited spatiotemporally, and the glacier volume and subsequent melt is poorly constrained, as is the englacial and subglacial hydrology. We postulate that the glacial melt must recharge beneath the glacier and/or through communication between shallow aquifers in glacial sediments and bedrock aquifers. Whether this process is occurring along the entire subsurface area of the ice-bedrock/alluvium/till contact, in localized pockets, and/or at the toe of the glacier is still unknown (and may remain unknown due to the logistical difficulty in sampling beneath glaciers), but may play an important role with regard to the isotopic signal that is recharging. The sampling window is still relatively narrow in high-alpine catchments due to seasonal inaccessibility and blockages to roads and trails during the melt season, therefore the spring/stream samples collected in this study represent a snapshot in time and do not take into account the possible seasonal change in isotopic values of the springs. As such, throughout time the fractional contribution of glacial ice may increase or decrease, but we predict that an overall decreasing trend is expected as the input from melting ice lessens. The end-member samples collected also represent a single point in space and time, and glacial ice, for example, contains an amalgamation of years of isotopic values. However, our sampling strategy has provided a robust range of possible isotopic values without the ability to collect ice cores at depth. Additionally, there are spatial limitations to the methods used here, as isotopes in glacial ice, snow, and rain vary as moisture moves across continents and mountainous terrain. If these methods are applied to a wider spatial extent than what is reasonable, erroneous conclusions may result. For example, a spring in MH and another in GNP appear to be glacially influenced, but these results should be taken with caution. In MH, the spring site in the bottom right-hand corner of the study area (figure 2(A)) was chosen as a test site because it is
located near the top of a local mafic stratovolcano that was thought to have its own local groundwater system separate from the MH system; however, the model predicted it was glacially influenced. In GNP, a spring located >20 km from a glacier (figure 2(G)) in a separate thrust fault near the southwest end of a large northeast-southwest trending lake (Lake McDonald) is also glacially influenced according to the model. Additional studies on a seasonal scale as well as long-term, continuous studies are needed to assess the temporal impact of glacial melt on mountain aquifers. This topic involves the intersection of hydrology, glaciology, and microbiology and has countless opportunities for further research.

Given the current rate of glacier recession and predictions for the future, many alpine glaciers will likely disappear within the century. This study shows that some of the meltwater stays in the mountain groundwater system; however, this does not imply that the mountain groundwater system has intrinsic or ‘built-in’ long-term sustainability. These findings indicate quite the opposite, that perennial flow from alpine springs and baseflow in headwater alpine streams is supported, at least partially in most cases and dominantly in others, by a transient source of recharge. Globally, nearly 670 million people live in high mountain regions, including indigenous peoples [49], with at least 1/6 of the world’s population depending on glaciers and seasonal snowpack for water [50]. Glacial runoff delivers vital water for communities and ecosystems, but if some of this water is being recharged and temporarily stored in the mountain block, then there is a lag time in delivery that is not being accurately considered in regional water studies or management strategies. Quantification of MBR could facilitate water conservation strategies and inform estimates of seasonal water supply as glaciers recede. Groundwater discharged as springs has been recognized as an important water source in mountain communities for centuries, but the long-term dependability of this source is now in question. Long-term monitoring of a few of the springs’ discharge or water level, in addition to seasonal isotopic and ecologic sampling would yield invaluable water resource data not only in MH and GNP, but in alpine, glaciated regions around the globe. Implementing level loggers (for hydrograph purposes), autosamplers (for geochemistry), or other passive sampling would likely be the easiest monitoring strategies, as these spring locations are typically difficult to access on a regular basis. Springs that have more defined, rather than diffuse, discharge sources and have a depth of at least a few centimeters are recommended. For future studies, it would be especially interesting to target high discharge springs or springs forming headwaters of societally important rivers and streams.

Changing climate and glacier recession over the past century is disturbing natural ecosystem function including freshwater species biodiversity. Glacier carbon supports downstream ecosystems and our data indicate glacier-fed springs/streams support distinct microbiota, but we do not know if the differences in microbial community structure observed here have impacts for invertebrate (and in extension, vertebrate) communities in alpine aquatic ecosystems [51, 52]. By emphasizing the importance and presence of groundwater dependent ecosystems and their connection to recharge sources, surface waters, and riparian zones, the emerging discipline of ecohydrogeology has potential to shed light many unresolved groundwater debates [24]. This study shows that glacial melt is critical to mountain aquifers and holds vital importance from both an ecological and water resource perspective, thereby highlighting the intricate connections between the cryosphere and deep hydrosphere.

Data availability statement

The SSU rRNA raw sequence read data have been deposited with links to BioProject accession number PRJNA629965 in the NCBI BioProject database (www.ncbi.nlm.nih.gov/bioproject/). Sampling site information, stable isotopic data, and mixing model results have been archived using Purdue PURR: Miller et al. (2021). Isotopic data and mixing model results of springs and streams in Mount Hood National Forest and Glacier National Park, USA. Purdue University Research Repository. DOI: https://doi.org/10.4231/PBEN-FT39. Photos and videos of springs sampled have been archived also using Purdue PURR: Miller (2021). Photos and videos documenting spring emergences and sampling locations in and around Mount Hood National Forest and Glacier National Park. Purdue University Research Repository. DOI: https://doi.org/10.4231/1R4N-RP33. Raw code for producing the mixing model results is available upon reasonable request from the corresponding author.

The data that support the findings of this study are openly available at the following URL/DOI: https://doi.org/10.4231/PBEN-FT39.

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