Evidence for microbial mediation of subseafloor nitrogen redox processes at Loihi Seamount, Hawaii

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Abstract

The role of nitrogen cycling in submarine hydrothermal systems is far less studied than that of other biologically reactive elements such as sulfur and iron. In order to address this knowledge gap, we investigated nitrogen redox processes at Loihi Seamount, Hawaii, using a combination of biogeochemical and isotopic measurements, bioenergetic calculations and analysis of the prokaryotic community composition in venting fluids sampled during four cruises in 2006, 2008, 2009 and 2013. Concentrations of NH₄⁺ were positively correlated to dissolved Si and negatively correlated to NO₃⁻/NO₂⁻, while NO₂⁻ was not correlated to NO₃⁻/NO₂⁻ dissolved Si or NH₄⁺. This is indicative of hydrothermal input of NH₄⁺ and biological mediation influencing NO₂⁻ concentrations. The stable isotope ratios of NO₃⁻ (δ¹⁵N and δ¹⁸O) was elevated with respect to background seawater, with δ¹⁸O values exhibiting larger changes than corresponding δ¹⁵N values, reflecting the occurrence of both production and reduction of NO₃⁻ by an active microbial community. δ¹⁵N-NH₄⁺ values ranged from 0‰ to +16.7‰, suggesting fractionation during consumption and potentially N-fixation as well. Bioenergetic calculations reveal that several catabolic strategies involving the reduction of NO₃⁻ and NO₂⁻ coupled to sulfide and iron oxidation could provide energy to microbes in Loihi fluids, while 16S rRNA gene sequencing of Archaea and Bacteria in the fluids reveals groups known to participate in denitrification and N-fixation. Taken together, our data support the hypothesis that microbes are mediating N-based redox processes in venting hydrothermal fluids at Loihi Seamount.

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Keywords: Hydrothermal vent; Nitrogen; Biogeochemistry; Isotopes; Bioenergetics; Subsurface biosphere; Geomicrobiology; Loihi

1. INTRODUCTION

Loihi is a model system for mid-plate hotspot magmatism. Hydrothermal activity at Loihi seamount is dominated by low-temperature vents emitting fluids up to ~70 °C with elevated concentrations of dissolved Fe(II), CO₂, CH₄ and NH₄⁺ (Gamo et al., 1987; Karl et al., 1989; Sedwick et al., 1992). In contrast to mid-ocean ridge
hydrothermal vents, hydrothermal fluids at Loihi are depleted in H₂S, making Loihi an excellent location to study microbial Fe-cycling (Emerson and Moyer, 2002; Glazer and Rouxel, 2009; Edwards et al., 2011).

Hydrothermal activity at Loihi is characterized by two modes of venting. At the summit, hydrothermal activity is currently present mostly in the Pele’s Pit crater, which is home to the Hiolo North area of venting around 1300 meters (m) below sea level, the Pohaku area around 1178 m depth and the Hiolo South area around 1274 m (Karl et al., 1989; Sedwick et al., 1992; Glazer and Rouxel, 2009; Jesser et al., 2015). These three areas are characterized by diffuse flow venting of warm hydrothermal fluids ~20–50 °C with iron-rich microbial mats found near the vent sites. The microbial mats at Loihi’s summit are generally dominated by members of the Zetaproteobacteria at sites with venting temperatures <40 °C, while increasing proportions of Epsilonproteobacteria are detected at sites with venting temperatures warmer than that (Moyer et al., 1994, 1995, 1998; Emerson and Moyer, 2002; Rassa et al., 2009).

Recently, a new type of hydrothermal activity was detected at the base of Loihi Seamount, at the site referred to as Ula Nui, located 5000 m deep at the base of the volcano. Ventiing at Ula Nui is characterized by ultra-diffuse venting, with a temperature anomaly only 0.2 °C above the ambient temperature of 1.7 °C (Edwards et al., 2011). This low temperature venting supports massive microbial mats that grow to >1 m tall and are largely dominated by Zetaproteobacteria.

In comparison to studies of sulfur redox processes in marine hydrothermal systems, there are far fewer studies of nitrogen redox processes. Recently, however, several studies have shown that genes involved in microbial nitrogen redox reactions are abundant in hydrothermal settings, including the presence of anaerobic ammonia oxidation (anammox) across a variety of hydrothermal settings (Byrne et al., 2009), nitrogen fixation genes (Mehta et al., 2009), and an inverse relationship between NH₄⁺ and NO₃⁻ concentrations of 0.28–5.56 μM and an inverse relationship between NH₄⁺ and NO₃⁻ (Karli et al., 1989; Sedwick et al., 1992), there have been no studies focusing on nitrogen (N) cycling. Here, we investigate nitrogen cycling processes at Loihi Seamount using a combination of biogeochemical and isotopic measurements, bioenergetic calculations and analysis of the prokaryotic community composition. While the microbial mats at Loihi have been well characterized (Moyer et al., 1994, 1995, 1998; Emerson and Moyer, 2002; Rassa et al., 2009; Edwards et al., 2011; Jesser et al., 2015), the microbiology of the venting fluids has not been previously described. Our analysis reveals the occurrence of several nitrogen redox transformations in Loihi subsurface fluids and sheds light on the putative microbial lineages associated with them.

2. SAMPLING AND ANALYTICAL METHODS

2.1. Sampling

Four cruises were conducted to Loihi Seamount: 22 September–10 October 2008 and 16 March–01 April 2013 aboard R/V Thomas G. Thompson and 11–27 October 2006 and 01–17 October 2009 aboard R/V Kilo Moana. We sampled hydrothermal fluid samples, labeled “Vent Fluids” in Table 1, from sites at Hiolo North (M31, M36, M39), Hiolo South (M34 and M38; previously named Loihau, renamed Hiolo South by Jesser et al., 2015), and Ula Nui. Areas and sites sampled are labeled in Fig. 1. Background seawater samples were collected away from venting in Pele’s Pit, Pit of Death, and at Ula Nui (Table 1). Non-buoyant hydrothermal plume samples, labeled “Water Column Profiles” in Table 1, were collected in Pele’s Pit and Pit of Death and during a Tow-Yo CTD cast south-west of Loihi’s summit (Bennett et al., 2011). In addition to these sites, which have been visited in previous studies of Loihi (Glazer and Rouxel, 2009; Edwards et al., 2011; Jesser et al., 2015), two new sites were discovered and sampled in the Hiolo South area (near Markers 34 and 38) during the 2009 expedition (Table 1). One new area of venting chimellets (small iron-oxide chimneys) was discovered between Markers 34 and 38 (labeled M34 → M38). The other new site was an approximately meter tall Fe-oxhydroxide chimney dubbed “Red Smoker”.

Hydrothermal vent samples destined for chemical analysis were collected from venting fissures in basalt rocks, ferruginous chimneys and a microbial mat (sample 477-MS-blue) using a titanium Major sampler deployed from ROV Jason II. The operation of the Major samplers for hydrothermal vent research has been described previously (Von Damm et al., 1985), as well as specifically for Loihi (Glazer and Rouxel, 2009). The Major sampler was placed directly in the venting orifice for rocky fissures and into the mouth of ferruginous chimneys. The ferruginous chimney structures are very delicate, therefore care was taken to place the snorkel of the Major sampler inside of the chimneys without causing the structure to collapse. For the mat sample collected with a Major sampler at Ula Nui, the sampling snorkel was pressed approximately 15 cm below the surface of the 1 m tall mat and triggered.

During the 2013 cruise, a newly designed microbial mat sampler (Breier et al., 2012) was used to specifically sample depth profiles within microbial mats. Briefly, the samplers consist of six 60-ml syringes arranged on a cassette for
Table 1
Composition of hydrothermal vent fluids, microbial mat samples (“-BM1-”), background seawater, and water column profiles collected from Loihi Seamount during 2008 (sample name begins with 3xx or 08xx-xx), 2009 (sample name begins with 4xx or 09xx-xx) and 2013 (sample name begins with 6xx). Units of measurement for biogeochemical measurements are μM, depth is in meters, bd = below detection, – = not measured.

<table>
<thead>
<tr>
<th>Site</th>
<th>Sample</th>
<th>Depth</th>
<th>Temp (°C)</th>
<th>NH$_4^+$</th>
<th>NO$_2$</th>
<th>NO$_3$ + NO$_2$</th>
<th>dSi</th>
<th>PO$_4^{3-}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vent fluids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hiolo North area</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M36</td>
<td>476-MS-blue</td>
<td>1303</td>
<td>35.6</td>
<td>2.615</td>
<td>–</td>
<td>0.82</td>
<td>–</td>
<td>3.00</td>
</tr>
<tr>
<td>M39</td>
<td>479-MS-black</td>
<td>1300</td>
<td>45.8</td>
<td>2.074</td>
<td>–</td>
<td>14.09</td>
<td>–</td>
<td>2.00</td>
</tr>
<tr>
<td>M39</td>
<td>482-MS-blue</td>
<td>1301</td>
<td>42.7</td>
<td>2.724</td>
<td>–</td>
<td>8.17</td>
<td>–</td>
<td>3.00</td>
</tr>
<tr>
<td>M39</td>
<td>482-MS-red</td>
<td>1301</td>
<td>42.7</td>
<td>2.291</td>
<td>–</td>
<td>3.19</td>
<td>–</td>
<td>3.20</td>
</tr>
<tr>
<td>M31</td>
<td>482-MS-black</td>
<td>1297</td>
<td>40.6</td>
<td>2.357</td>
<td>–</td>
<td>1.52</td>
<td>–</td>
<td>3.20</td>
</tr>
<tr>
<td>M31</td>
<td>476-MS-red</td>
<td>1301</td>
<td>43</td>
<td>2.815</td>
<td>–</td>
<td>1.27</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>M31</td>
<td>675-MS-black2</td>
<td>1300</td>
<td>41.3</td>
<td>2.278</td>
<td>0.095</td>
<td>1.55</td>
<td>500.6</td>
<td>4.44</td>
</tr>
<tr>
<td>M31</td>
<td>672-MS-yellow</td>
<td>1300</td>
<td>40.7</td>
<td>2.096</td>
<td>0.093</td>
<td>1.86</td>
<td>218.6</td>
<td>3.70</td>
</tr>
<tr>
<td>M31</td>
<td>675-MS-red2</td>
<td>1300</td>
<td>41.3</td>
<td>2.122</td>
<td>0.142</td>
<td>1.51</td>
<td>464.6</td>
<td>3.82</td>
</tr>
<tr>
<td>Upper M31</td>
<td>674-MS-yellow</td>
<td>1300</td>
<td>–</td>
<td>1.584</td>
<td>0.493</td>
<td>6.86</td>
<td>310.6</td>
<td>1.06</td>
</tr>
<tr>
<td>47 deg site</td>
<td>672-MS-black</td>
<td>1298</td>
<td>47.1</td>
<td>2.721</td>
<td>bd</td>
<td>1.05</td>
<td>270.6</td>
<td>2.35</td>
</tr>
<tr>
<td>Directly above M31, near M39, same site as 676-MS-white, in orifice</td>
<td>676-MS-white</td>
<td>1300</td>
<td>–</td>
<td>1.322</td>
<td>bd</td>
<td>22.30</td>
<td>286.6</td>
<td>0.26</td>
</tr>
<tr>
<td>Texture Garden (between M31 &amp; M39)</td>
<td>676-MS-black</td>
<td>1298</td>
<td>30.8</td>
<td>3.032</td>
<td>0.236</td>
<td>11.82</td>
<td>352.6</td>
<td>2.72</td>
</tr>
<tr>
<td><strong>Hiolo South area</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M38</td>
<td>675-MS-white</td>
<td>1274</td>
<td>43.3</td>
<td>2.408</td>
<td>bd</td>
<td>2.91</td>
<td>432.6</td>
<td>6.64</td>
</tr>
<tr>
<td>M38</td>
<td>675-MS-yellow</td>
<td>1274</td>
<td>42.4</td>
<td>2.647</td>
<td>bd</td>
<td>2.67</td>
<td>522.6</td>
<td>6.40</td>
</tr>
<tr>
<td>M34</td>
<td>675-MS-black</td>
<td>1272</td>
<td>47.4</td>
<td>1.925</td>
<td>0.215</td>
<td>4.53</td>
<td>700.6</td>
<td>6.22</td>
</tr>
<tr>
<td>M34</td>
<td>675-MS-yellow2</td>
<td>1270</td>
<td>48.2</td>
<td>2.660</td>
<td>bd</td>
<td>1.38</td>
<td>488.6</td>
<td>3.82</td>
</tr>
<tr>
<td>M34</td>
<td>675-MS-red</td>
<td>1272</td>
<td>47.4</td>
<td>0.705</td>
<td>bd</td>
<td>25.21</td>
<td>256.6</td>
<td>3.33</td>
</tr>
<tr>
<td>M34</td>
<td>675-MS-white2</td>
<td>1270</td>
<td>48.1</td>
<td>2.508</td>
<td>bd</td>
<td>1.164</td>
<td>450.6</td>
<td>6.09</td>
</tr>
<tr>
<td>M34</td>
<td>479-MS-blue</td>
<td>1273</td>
<td>50.1</td>
<td>4.249</td>
<td>–</td>
<td>7.22</td>
<td>–</td>
<td>4.50</td>
</tr>
<tr>
<td>M34</td>
<td>483-MS-white</td>
<td>1273</td>
<td>50.7</td>
<td>2.398</td>
<td>–</td>
<td>24.39</td>
<td>–</td>
<td>2.90</td>
</tr>
<tr>
<td>M34</td>
<td>476-MS-black</td>
<td>1272</td>
<td>41.8</td>
<td>3.655</td>
<td>–</td>
<td>1.37</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>M34</td>
<td>373-MS-red</td>
<td>1271</td>
<td>51.5</td>
<td>7.306</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2.39</td>
</tr>
<tr>
<td>M34</td>
<td>373-MS-black</td>
<td>1271</td>
<td>51.5</td>
<td>3.606</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1.26</td>
</tr>
<tr>
<td>M34, few cm into mat</td>
<td>675-BM1-C2</td>
<td>1271</td>
<td>–</td>
<td>2.128</td>
<td>0.166</td>
<td>31.73</td>
<td>426.6</td>
<td>1.12</td>
</tr>
<tr>
<td>M34, few cm into mat</td>
<td>675-BM1-C4</td>
<td>1271</td>
<td>–</td>
<td>2.536</td>
<td>0.149</td>
<td>22.68</td>
<td>448.6</td>
<td>1.47</td>
</tr>
<tr>
<td>M34, ~1–2 cm into diffuse flow orifice with mat surrounding orifice, same area as C4</td>
<td>675-BM1-C6</td>
<td>1271</td>
<td>–</td>
<td>3.088</td>
<td>0.347</td>
<td>26.37</td>
<td>432.6</td>
<td>2.73</td>
</tr>
<tr>
<td>M34-&gt;M38</td>
<td>483-MS-black</td>
<td>1276</td>
<td>47.4</td>
<td>0.754</td>
<td>–</td>
<td>30.82</td>
<td>–</td>
<td>0.90</td>
</tr>
<tr>
<td>M38</td>
<td>479-MS-white</td>
<td>1274</td>
<td>42</td>
<td>3.114</td>
<td>–</td>
<td>8.87</td>
<td>–</td>
<td>3.00</td>
</tr>
<tr>
<td>Red Smoker</td>
<td>483-MS-blue</td>
<td>1254</td>
<td>47.4</td>
<td>2.951</td>
<td>–</td>
<td>13.17</td>
<td>–</td>
<td>2.90</td>
</tr>
<tr>
<td><strong>Pohaku area</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>M57</td>
<td>368-MS-red</td>
<td>1178</td>
<td>26.7</td>
<td>4.090</td>
<td>–</td>
<td>9.03</td>
<td>689.6</td>
<td>2.31</td>
</tr>
<tr>
<td>M57</td>
<td>368-MS-black</td>
<td>1178</td>
<td>28.3</td>
<td>2.808</td>
<td>–</td>
<td>20.70</td>
<td>605.6</td>
<td>0.73</td>
</tr>
<tr>
<td>M57</td>
<td>476-MS-white</td>
<td>1178</td>
<td>24</td>
<td>2.431</td>
<td>–</td>
<td>21.13</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>M57</td>
<td>671-MS-white</td>
<td>1177</td>
<td>25.9</td>
<td>4.211</td>
<td>0.333</td>
<td>17.70</td>
<td>160.6</td>
<td>3.21</td>
</tr>
<tr>
<td>M57</td>
<td>671-MS-red</td>
<td>1177</td>
<td>25.9</td>
<td>4.235</td>
<td>0.124</td>
<td>30.01</td>
<td>210.6</td>
<td>0.45</td>
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<tr>
<td><strong>Ula Nui area</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ula Nui Mat</td>
<td>477-MS-blue</td>
<td>4984</td>
<td>2.8</td>
<td>1.555</td>
<td>–</td>
<td>25.27</td>
<td>–</td>
<td>4.10</td>
</tr>
<tr>
<td>Ula Nui ‘orange mat 1’ surface</td>
<td>673-BM1-A2</td>
<td>4983</td>
<td>–</td>
<td>0.608</td>
<td>0.185</td>
<td>21.67</td>
<td>303</td>
<td>3.07</td>
</tr>
<tr>
<td>Ula Nui ‘black mat 1’ surface</td>
<td>673-BM1-A3</td>
<td>4983</td>
<td>–</td>
<td>0.511</td>
<td>bd</td>
<td>30.25</td>
<td>231</td>
<td>2.92</td>
</tr>
</tbody>
</table>

(continued on next page)
which the syringe being sampled and speed of sampling is driven by a motor to allow for precise sampling of mats at specific depths. For samples destined for chemical analysis, a 0.2 l m syringe tip filter was placed inline so that the sample was filtered in situ as it was drawn into the syringe.

Background seawater samples were collected away from venting sites using Niskin bottles attached to the side of ROV Jason II. In one case, a Major sampler was fired away from venting to collect a background sample, and in another case, a single syringe of the mat sampler was used for background seawater. Water column profiles were conducted and hydrothermal plume samples were targeted and collected using niskin bottles on a CTD rosette. The plume emanating from Loihi’s summit was detected using transmissometry, as detailed in Bennett et al. (2011).

All samples for chemical analysis were either filtered and then frozen (all samples from 2008 and 2013, CTD samples in 2009) or frozen immediately and filtered upon thawing before analysis (2009). For those filtered prior to freezing, samples from Major samplers were filtered through a 0.20 l m pore size syringe tip filter placed inline with the outlet of the Major sampler. Water column profile samples were filtered as they exited the niskin bottles with 0.20 l m pore size, 47 mm diameter Supor filters (Pall) in PFA filter holders (Cole-Parmer). All other samples were filtered using syringe tip filters on 60 ml syringes. Samples were stored in sterile polypropylene containers until analysis. An aliquot of sample was used to rinse the containers and discarded prior to filling the containers with sample.

Four diffuse flow hydrothermal fluid samples and two background seawater samples were sampled for microbial community analysis during the 2006 cruise (Table 2). LoihiPP1, 2, 4, 5 and 6 were sampled using the pelagic pump on the ROV Jason II during dives J2-241, J2-242, J2-243, J2-245 and J2-246, respectively. A hose with a coarse mesh filter at the sampling point was placed in venting diffuse fluids (LoihiPP1, 2, 5 and 6), and ~50 L was then filtered through a Steripak-GP 0.22 l m pore size filter. The filter was frozen at −80 °C upon retrieval of the vehicle. One background seawater sample (LoihiPP4) was collected in the same manner (~125 L filtered through a Steripak-GP) while the ROV was in the water column in Pele’s Pit. Another background seawater sample, LoihicTD03, was collected with a CTD rosette in Pele’s Pit and then 10 L filtered through a Sterivex GP 0.22 l m pore size filter.
2.2. Chemistry analytical methods

Fluid temperatures were measured by placing the temperature probe on ROV Jason II into the venting orifice or chimney. NO$_3$ + NO$_2$ (hereafter referred to as NO$_3$ + NO$_2$) and NO$_2$/O$_2$ were measured using the chemiluminescent method after reduction to NO by hot, acidic vanadium (NO$_2$ + NO$_3$) or potassium iodide (NO$_2$/O$_2$) (Garside, 1982) with a detection limit of <0.010 μM. NH$_4$+ was measured colorimetrically via the phenol-hypochlorite method (Grasshoff et al., 1999) with a 5 cm cell in a Shimadzu UV-1601 spectrophotometer onboard the RIV Thompson (2008) or using the fluorescence method (Holmes et al., 1999) post-cruise (2009 and 2013). The detection limit for NH$_4$+ by both methods is 0.030 μM. Spiked samples were within 5% of expected values or better for both methods.

Dissolved inorganic phosphorus (P$_i$) and dissolved silica (dSi) were measured using colorimetric methods, with detection limits of 0.030 μM for P$_i$ and 0.30 μM for dSi (Gieskes et al., 1991; Grasshoff et al., 1999).

Table 2
Basic data for samples from which DNA sequences were obtained.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Date collected</th>
<th>Site</th>
<th>Depth (m)</th>
<th># V6 Tags</th>
<th>#V6 tags after removing background OTUs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vent fluids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LoihiPP1-bac</td>
<td>27 Oct 2006</td>
<td>Marker 34</td>
<td>1272</td>
<td>11,707</td>
<td>1855</td>
</tr>
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<td>LoihiPP1-arc</td>
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<td>21,806</td>
<td>3901</td>
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<tr>
<td>LoihiPP2-bac</td>
<td>31 Oct 2006</td>
<td>Hiolo North Area</td>
<td>1302</td>
<td>14,035</td>
<td>6947</td>
</tr>
<tr>
<td>LoihiPP2-arc</td>
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<td>Marker 31</td>
<td>1301</td>
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<td></td>
<td></td>
<td>19,045</td>
<td>1969</td>
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<tr>
<td>LoihiPP6-bac</td>
<td>07 Nov 2006</td>
<td>Ula Nui</td>
<td>4987</td>
<td>16,200</td>
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<tr>
<td>LoihiPP6-arc</td>
<td></td>
<td></td>
<td></td>
<td>13,961</td>
<td>336</td>
</tr>
<tr>
<td><strong>Background seawater</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LoihiCTD03-bac</td>
<td>31 Oct 2006</td>
<td>Pele’s Pit</td>
<td>1100</td>
<td>19,108</td>
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</tr>
<tr>
<td>LoihiCTD03-arc</td>
<td></td>
<td></td>
<td></td>
<td>14,790</td>
<td>–</td>
</tr>
<tr>
<td>LoihiPP4-bac</td>
<td>02 Nov 2006</td>
<td>Pele’s Pit</td>
<td>1717</td>
<td>18,682</td>
<td>–</td>
</tr>
<tr>
<td>LoihiPP4-arc</td>
<td></td>
<td></td>
<td></td>
<td>15,336</td>
<td>–</td>
</tr>
</tbody>
</table>

Fig. 1. Map of Loihi Seamount, with sampling sites indicated. Inset at the bottom of the left panel indicates the location of Loihi in the Pacific Ocean. Rectangle at the top of the left panel highlights the location of the area in the right panel. Sites marked by a yellow circle in the right panel are in Hiolo North and sites marked by a yellow star are in Hiolo South, as indicated by the key at right.
To determine if vent fluid chemistry differed between Hiolo North, Hiolo South and Pohaku, one-way analysis of variance (ANOVA) was calculated with Tukey’s post hoc pairwise comparison for hydrothermal vent fluid temperature and all chemical variables measured using JMP Pro 10 (SAS Institute, Inc.). Correlations between the same chemical variables across all samples were determined using Kendall’s τ correlation.

### 2.3. Isotopic measurements

Nitrate N and O stable isotope ratios (\(^{15}\text{N}/^{14}\text{N}\) and \(^{18}\text{O}/^{16}\text{O}\), respectively) were measured using the denitrifier method (Sigman et al., 2001; Casciotti et al., 2002), in which sample NO\(_3\) is quantitatively converted to N\(_2\)O using a lab-grown denitrifying bacterium before being extracted and purified on a purge and trap system similar to that previously described in McIlvin and Casciotti (2010). Isotope ratios are expressed using standard delta notation where \(\delta\) is normalized to international isotopic reference standards: IAEA-D\(_2\) for \(\delta^{15}\text{N}\) and USGS-26 (Casciotti et al., 2002; McIlvin and Casciotti, 2010), with a typical reproducibility of 0.2‰ and 0.4‰ for \(\delta^{15}\text{N}\) and \(\delta^{18}\text{O}\), respectively. Isotope ratios are reported relative to N\(_2\) in air as reference, while \(\delta^{15}\text{N}\) of NO\(_3\) was removed by sulfamic acid addition (Granger and Sigman, 2009) prior to isotopic analysis of NO\(_3\). Isotope ratios were measured on an IsoPrime100 (Elementar, Inc.) and corrections for drift, size and fractionation of O isotope ratios were measured on an IsoPrime100 (Elementar, Inc.). Where detected, NO\(_2\) was oxidized to NO\(_3\) via persulfate-converted sample provides \(\delta^{15}\text{N}\) of (NO\(_3\)+NO\(_2\)-NH\(_4\)) while a parallel sample without persulfate oxidation step yields \(\delta^{15}\text{N}\) of (NO\(_2\)+NO\(_3\)).

The high energy scenario was generated using the revised-HKF equations of Sigman et al., 2009) prior to isotopic analysis of NO\(_3\). Isotope ratios were measured on an IsoPrime100 (Elementar, Inc.) and corrections for drift, size and fractionation of O isotope ratios were measured on an IsoPrime100 (Elementar, Inc.). Where detected, NO\(_2\) was oxidized to NO\(_3\) via persulfate-converted sample provides \(\delta^{15}\text{N}\) of (NO\(_3\)+NO\(_2\)-NH\(_4\)) while a parallel sample without persulfate oxidation step yields \(\delta^{15}\text{N}\) of (NO\(_2\)+NO\(_3\)). Isotopic composition of the NH\(_4\) pool was calculated by mass balance to report \(\delta^{15}\text{N}\) of NH\(_4\) values, which were normalized to international isotopic reference standards: IAEA-N1 (\(\delta^{15}\text{N} = 0.5\%e\)), USGS-25 (\(\delta^{15}\text{N} = -29.4\%e\)) and USGS-26 (\(\delta^{15}\text{N} = 52.9\%e\)).

### 2.4. Bioenergetic calculations

Values of the energy densities of the rth reaction per kg of water, \(E_r\), are calculated using (LaRowe et al., 2014):

\[
E_r = \frac{\Delta G_r}{v_i [i]}
\]

where \(v_i\) and \([i]\) stand for the stoichiometric coefficient and molal concentration, respectively, of the \(i\)th limiting electron donor or acceptor. Because either the electron donor or acceptor will be a limiting reactant per volume of fluid, the concentration and stoichiometric coefficient of this limiting nutrient were used for values of \(v_i\) and \([i]\) in Eq. (1). In order to carry out these calculations, the activities of all reactants and products were held constant. In effect, this is an instantaneous snapshot of the total amount of Gibbs energy contained in a kg of water for a particular reaction. Because the prevailing physiochemical conditions at the sample sites vary with time, Gibbs energy densities were calculated for high and low energy scenarios in order to capture the natural variability of hydrothermal vents at Loihi. The high energy scenario was generated using the highest concentrations of reactants and lowest concentrations of product species at each sample site. Conversely, the low energy scenario used the lowest concentrations of reactants and highest concentrations of product species at each sample site.

Values of \(\Delta G_r\) are calculated using

\[
\Delta G_r = -RT \ln \frac{K_r}{Q_r}
\]

where \(K_r\) and \(Q_r\) refer to the equilibrium constant and reaction quotient of the reaction, respectively, \(R\) represents the gas constant, and \(T\) denotes temperature in Kelvin. Values of \(K_r\) were calculated using the revised-HKF equations of Sigman et al., 2009) prior to isotopic analysis of NO\(_3\). Isotope ratios were measured on an IsoPrime100 (Elementar, Inc.) and corrections for drift, size and fractionation of O isotope ratios were measured on an IsoPrime100 (Elementar, Inc.). Where detected, NO\(_2\) was oxidized to NO\(_3\) via persulfate-converted sample provides \(\delta^{15}\text{N}\) of (NO\(_3\)+NO\(_2\)-NH\(_4\)) while a parallel sample without persulfate oxidation step yields \(\delta^{15}\text{N}\) of (NO\(_2\)+NO\(_3\)). Isotopic composition of the NH\(_4\) pool was calculated by mass balance to report \(\delta^{15}\text{N}\) of NH\(_4\) values, which were normalized to international isotopic reference standards: IAEA-N1 (\(\delta^{15}\text{N} = 0.5\%e\)), USGS-25 (\(\delta^{15}\text{N} = -29.4\%e\)) and USGS-26 (\(\delta^{15}\text{N} = 52.9\%e\)).

### Table 3

<table>
<thead>
<tr>
<th>Reactions considered in this study.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Iron oxidation with nitrite reduction</strong></td>
</tr>
<tr>
<td>01. 2Fe(^{2+}) + NO(_3) + 3H(_2)O → 2Fe(^{3+}) + NO(_2)+4H(_2)O</td>
</tr>
<tr>
<td>02. 5Fe(^{2+}) + NO(_3) + 7H(_2)O → 5Fe(^{3+}) + 0.5N(<em>2)+9H(</em>+)</td>
</tr>
<tr>
<td>03. 8Fe(^{2+}) + NO(_3) + 13H(_2)O → 8Fe(^{3+}) + NH(<em>4)+14H(</em>+)</td>
</tr>
<tr>
<td>04. 3Fe(^{2+}) + NO(_3) + 4H(_2)O → 3Fe(^{3+}) + 0.5N(<em>2)+10H(</em>+)</td>
</tr>
<tr>
<td>05. 6Fe(^{2+}) + NO(_3) + 10H(_2)O → 6Fe(^{3+}) + NH(<em>4)+10H(</em>+)</td>
</tr>
<tr>
<td><strong>Methane oxidation with nitrate or nitrite reduction</strong></td>
</tr>
<tr>
<td>06. CH(_4) + 4NO(_3) → CO(_2) + 4NO(_2)+2H(_2)O</td>
</tr>
<tr>
<td>07. CH(_4) + 8NO(<em>3) + 8H(</em>+) → 5CO(_2) + 4N(_2)+14H(_2)O</td>
</tr>
<tr>
<td>08. CH(_4) + NO(<em>3) + 2H(</em>+) → CO(_2) + NH(_4)+H(_2)O</td>
</tr>
<tr>
<td>09. 3CH(_4) + 4NO(<em>3) + 8H(</em>+) → 3CO(_2) + 4N(_2)+10H(_2)O</td>
</tr>
<tr>
<td>10. 3CH(_4) + 4NO(<em>3) + 8H(</em>+) → 3CO(_2) + 4NH(_4)+2H(_2)O</td>
</tr>
<tr>
<td><strong>Sulfide oxidation with nitrate or nitrite reduction</strong></td>
</tr>
<tr>
<td>11. 5H(_2)S + 8NO(_3) → 5SO(_4)(^2-) + 4N(<em>2)+2H(</em>+)+4H(_2)O</td>
</tr>
<tr>
<td>12. H(_2)S + NO(_3) + H(_2)O → SO(_4)(^2-) + NH(_4)</td>
</tr>
<tr>
<td>13. 3H(_2)S + 8NO(<em>3) + 2H(</em>+) → 3SO(_4)(^2-) + 4N(_2)+4H(_2)O</td>
</tr>
<tr>
<td>14. 3H(_2)S + 4NO(_3) + 4H(<em>2)O + 2H(</em>+) → 3SO(_4)(^2-) + 4NH(_4)</td>
</tr>
<tr>
<td><strong>Anammox</strong></td>
</tr>
<tr>
<td>15. NH(_4)+NO(_2) → N(_2)+2H(_2)O</td>
</tr>
</tbody>
</table>

**Ammonium or nitrite oxidation**

16. NH\(_4\)+1.5O\(_2\) → NO\(_2\)+H\(_2\)O + 2H\(_+\) |
17. NO\(_2\)+0.5O\(_2\) → NO\(_3\)
Table 4
Temperatures and concentrations (µM) of select species used in the thermodynamic calculations at the indicated samples sites. The concentrations of species used in calculations but not measured here or specifically at the sites sampled here are as follows: CH₄ (aq) = 177 nM, average of values from (Karl et al., 1989); pH = 6.2 average of values taken from (Glazer and Rouxel, 2009); SO₄²⁻ = 28 mM (seawater); N₂ (aq) = 0.51 mM (equilibrium with N₂(g) in atmosphere); CO₂ (aq) 18 mM (Karl et al., 1989); O₂ (aq) = 4 µM (this is a nominal microaerobic value).

<table>
<thead>
<tr>
<th>Site</th>
<th>T, °C</th>
<th>NO₂⁻</th>
<th>NO₃⁻</th>
<th>NH₄⁺</th>
<th>Fe²⁺</th>
<th>HS⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hiolo South</td>
<td>41.8–51.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.144–0.347</td>
<td>1.16–31.70</td>
<td>0.754–7.506</td>
<td>346–648&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.6–25.2&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pohaku</td>
<td>24.0–28.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.124–0.333</td>
<td>9.03–41.04</td>
<td>2.430–4.235</td>
<td>507–773&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.0&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hiolo North</td>
<td>25.7–27.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.093–0.493</td>
<td>0.82–22.30</td>
<td>1.320–3.030</td>
<td>117–799&lt;sup&gt;c&lt;/sup&gt;</td>
<td>18.5&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> From values reported in Table 1.
<sup>b</sup> Calculated from [NO₃⁻] = [NO₃⁺ + NO₂⁻] – [NO₂⁻] where values of [NO₃⁺ + NO₂⁻] – [NO₂⁻] are taken from Table 1.
<sup>c</sup> Glazer and Rouxel (2009).
<sup>d</sup> Nominal value.
<sup>e</sup> Assumed to be the same as bottom water.
<sup>f</sup> Edwards et al. (2011).

\[ Q_r = \prod_i a_i^{v_i}, \quad (3) \]

where \( a_i \) stands for the activity of the \( i \)th species and \( v_i \) corresponds to the stoichiometric coefficient of the \( i \)th species in the reaction of interest. Values of \( a_i \) are related to the concentration of the \( i \)th species, \( C_i \), through

\[ a_i = \gamma_i \left( \frac{C_i}{C_i^0} \right)^{v_i} \quad (4) \]

where \( \gamma_i \) stands for the activity coefficient of the \( i \)th species and \( C_i^0 \) refers to the concentration of the \( i \)th species under standard state conditions, which is taken to be equal to one molal referenced to infinite dilution. Values of \( \gamma_i \) were in turn computed as a function of temperature and ionic strength using an extended version of the Debye-Hückel equation (Helgeson, 1969). The reactions chosen to represent the catabolic potential of nitrogen-processing microbial communities at Loihi are comprised of electron donors (EDs) and electron acceptors (EAs) that are known to be present at this site (Table 3). Concentrations of NO₅, NO₂, NH₄⁺ used in these calculations are reported in the current study, and the concentrations of other species, such as Fe²⁺ and HS⁻, have been taken from other studies that have focused on the same sample sites (Glazer and Rouxel, 2009; Edwards et al., 2011); the data used is presented in Table 4.

2.5. DNA extraction, sequencing and data processing

Environmental DNA from diffuse flow fluids and background seawater was extracted using previously described methods (Sogin et al., 2006). Polymerase chain reaction of the V6 hypervariable region of the small subunit (SSU) rRNA gene for Bacteria and Archaea, followed by 454 pyrosequencing of the amplicons, was carried out as described previously for all diffuse flow and background seawater samples (Huber et al., 2007). Basic metadata for the samples used for pyrosequencing is given in Table 2.

Obtained sequences were run through the VAMPS pipeline (http://vamps.mbl.edu), which removed sequences with any N’s and trimmed primers, requiring an absolute match to the sequencing primers. Phylogenetic affiliations of the tag sequences (hereafter referred to as pyrotags) were identified using the Global Alignment for Sequence Taxonomy (GAST) method (Huse et al., 2008) for all samples. For pyrotags designated “unknown” by GAST, each individual sequence was submitted to the RDP classifier with the bootstrap parameter set to 80% (Cole et al., 2009). If the sequence was not assigned to the domain Bacteria for sequences obtained using bacterial primers, or Archaea for sequences obtained using archael primers, it was removed from further analysis. Operational taxonomic units, defined at the 97% similarity cutoff, were determined using the software package Mothur (Schloss et al., 2009) with the pre.cluster command, which preclusters at a 1% difference level (one bp difference for the V6 tags used here) using modified single-linkage (Huse et al., 2010) and the average neighbor method. To concentrate on operational taxonomic units (OTUs) present only in diffuse fluids, we removed from our samples any OTUs (defined at the 97% similarity cutoff) that were present in the two Loihi seawater samples (LoihiCTD03 and LoihiPP4) using the remove.otus command in mothur.

Raw sequence data is deposited in the NCBI SRA under Bioproject PRJNA109379. Quality-controlled trimmed reads can be found at vamps.mbl.edu under projects KCK_SMT_Av6 and KCK_SMT_Bv6.

3. RESULTS

3.1. Bulk chemistry

Hydrothermal venting at Loihi is most active near the Pele’s Pit crater. The mean temperature of the venting fluids in the Hiolo South area was ~47 °C, ~7 °C higher than the Hiolo North vents (Table 1). NH₄⁺ concentrations were always elevated in comparison to background seawater.
Fig. 2. Depth profiles of dSi, NO₃, NO₂ and NH₄⁺ within two microbial mats at Ula Nui. (A) Mat sampler collecting fluids from the surface of mat C5. Mat D6 can be seen to the left of the photograph. (B) Mat sampler collecting fluids at a depth of 15 cm in mat C5. (C) Depth profile in mat C5. (D) Depth profile in mat D6.

(<0.03 μM), ranging from 1.1 μM to 3.0 μM in the Hiolo North area, ~0.7 μM lower, on average, than those measured in the Hiolo South area (range 0.7–7.5 μM). Pohaku/M57, located on the outside rim of Pele’s Pit (Fig. 1), emits end-member fluids with a mean temperature of 26 °C and NH₄⁺ concentrations from 2.4 to 4.2 μM. The diffuse venting and background seawater samples in the Pit of Death contained elevated NH₄⁺ (0.2 μM) compared to typical deep ocean waters (<0.03 μM, samples 0801-21 and 0901-21, Table 1) in 2008, but this site was found to be inactive in 2009 and not sampled again. NO₃ + NO₂ concentrations ranged widely at both Hiolo North and the Hiolo South area, but were higher, on average, in the Hiolo South area, and all samples were generally much lower than background seawater (~41 μM). NO₂⁻ ranged from below detection up to 0.5 μM at various sites in the Hiolo South, Hiolo North and Pohaku areas. P_i was variable, ranging from 0.3 μM, approximately an order of magnitude less than background seawater, to 6.6 μM, approximately twice background seawater.

Loihi seamount is home to abundant ferruginous microbial mats (Karl et al., 1988; Emerson and Moyer, 2002). Concentrations of Fe²⁺ and oxygen are known to be variable from the surface to the deeper parts of these mats; oxygen decreases from saturation to below detection by 10 cm below the mat surface and dissolved Fe²⁺ increases continuously from 40 μM at the surface of the mat to ~120 μM at 70 cm below mat surface (Edwards et al., 2011). The interstitial space in these mats is comprised of a mix of background seawater and hydrothermal fluids from either the nearest orifice, as is the case with samples collected from the caldera, or from the bottom of the mat, as is the case with the mounds sampled at Ula Nui (Fig. 1). Samples obtained from a few cm below the surface of mats located at M34 all had elevated NH₄⁺, NO₂⁻ and dSi compared to background seawater concurrent with reduced concentrations NO₃ + NO₂ (Table 1). Sampling at the surface of four microbial mats at the ultra-diffuse venting Ula Nui site, known for meter tall nontronite laden mats (Edwards et al., 2011), revealed similar patterns. Additional information was gained from vertical profiling of two of these mats, which revealed increasing NH₄⁺ and dSi and decreasing NO₂⁻ with depth (Fig. 2). Mat C5 also had increasing NO₂⁻ with depth while NO₃⁻ was below detection in mat D6, which was located only 20 cm away. The gradients in the top 5 cm were steeper in mat C5 than mat D6.

In hydrothermal vent fluids from Loihi, Mg remains close to background seawater, unlike high temperature hydrothermal venting (Karl et al., 1989; Sedwick et al., 1992; Glazer and Rouxel, 2009). Therefore, concentrations of dSi are used as a conservative tracer of Loihi hydrothermal vent fluids because they are elevated compared to background and mix conservatively with deep ocean water. NO₃⁻ does not show a relationship to dSi (Fig. 3C), while NH₄⁺ is positively correlated to dSi (Fig. 3A). Two samples from M57 collected in 2013 had anomalously high dSi and are outliers to the trendline although believed to be accurate. NO₃⁻ + NO₂⁻ and NH₄⁺ are negatively correlated (Fig. 3B), as has been noted before (Karl et al., 1989; Sedwick et al., 1992). The linear relationships between both NH₄⁺ vs dSi and NO₃⁻ + NO₂⁻ vs NH₂⁺ are variable dependent on the year of sampling, including data from previous studies (Karl et al., 1989; Sedwick et al., 1992) (Fig. 3A and B).

One-way ANOVA was used to statistically compare the end-member fluid data (Table 5) from Hiolo North, Hiolo South and Pohaku. Hydrothermal fluid temperatures at the three areas in and around Pele’s Pit are significantly different at each area (p < 0.001), while NO₃⁻ + NO₂⁻ concentrations are significantly different between Pohaku and Hiolo.
North ($p = 0.0114$), and $P_i$ concentrations are significantly different between Hiolo South and Pohaku ($p = 0.0283$). $NH_4^+$ and dSi concentrations were not significantly variable between any of the three Pele’s Pit sites.

While linear regressions are stronger when each year is considered independently (Fig. 3), significant correlations remain even when pooling all data points from both this work and earlier studies (Karl et al., 1989; Sedwick et al., 1992), as shown in Table 5. Significant positive correlations exist between dSi and $NH_4^+$, while significant negative correlations exist between dSi and $NO_3^- + NO_2^-$, between $NO_3^- + NO_2^-$ and temperature, between $NH_4^+$ and $NO_3^- + NO_2^-$, between $NO_2^- / CO$ and temperature and between $P_i$ and $NO_3^- + NO_2^-$.

### 3.2. Stable isotope measurements

Background seawater from depths of ~1100 m (near Pele’s Pit) had $\delta^{15}N_{NO_3}$ of +6.3‰ and $\delta^{18}O_{NO_3}$ of +3.2‰ (Table 6). Low-temperature vent fluid samples (up to ~45 °C) collected from Pele’s Pit generally exhibited increasing isotope ratios with decreasing concentrations of $NO_3^-$ (Fig. 4a), with $\delta^{15}N$ ranging from +5.8 up to +11.5‰ and $\delta^{18}O$ from +4.0 up to +18.0‰. The changes in $\delta^{18}O_{NO_3}$ values were notably larger than the corresponding changes in $\delta^{15}N_{NO_3}$ values with respect to seawater (Fig. 4b), consistent with active cycling of N (see below). Vent plume samples collected from Pele’s Pit in 2009 showed $NO_3^-$ isotopic compositions that were largely indis-
Table 6
Isotopic composition for vent fluids, background seawater and water column profiles from Loihi Seamount. Temperature and nutrient data are as reported in Table 1. For some samples, $\delta^{15}$N-NH$_4$ could not be calculated because the mass balance based calculations yielded errors too large to report; these are labeled *. Isotopic composition for samples with no error reported were calculated a single time due to low sample volume.

<table>
<thead>
<tr>
<th>Site</th>
<th>Sample</th>
<th>Temp (°C)</th>
<th>NH$_4$</th>
<th>$\delta^{15}$N-NH$_4$</th>
<th>NO$_2$</th>
<th>NO$_3$ + NO$_2$</th>
<th>$\delta^{15}$N-NO$_3$</th>
<th>$\delta^{18}$O-NO$_3$</th>
<th>dSi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vent fluids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M31</td>
<td>675-MS-black2</td>
<td>41.3</td>
<td>2.278</td>
<td>7.5</td>
<td>0.095</td>
<td>1.55</td>
<td>9.6</td>
<td>12.9</td>
<td>500.6</td>
</tr>
<tr>
<td>M31</td>
<td>672-MS-yellow</td>
<td>40.7</td>
<td>2.096</td>
<td>14.0 ± 1.3</td>
<td>0.093</td>
<td>1.86</td>
<td>8.7</td>
<td>16.1</td>
<td>218.6</td>
</tr>
<tr>
<td>M31</td>
<td>675-MS-red2</td>
<td>41.3</td>
<td>2.122</td>
<td>5.5 ± 1.1</td>
<td>0.142</td>
<td>1.51</td>
<td>11.5</td>
<td>15.2</td>
<td>464.6</td>
</tr>
<tr>
<td>M39</td>
<td>674-MS-black</td>
<td>25.7</td>
<td>1.122</td>
<td>*</td>
<td>0.259</td>
<td>16.34</td>
<td>6.4 ± 0.5</td>
<td>3.1 ± 1.1</td>
<td>268.6</td>
</tr>
<tr>
<td>Upper M31</td>
<td>674-MS-yellow</td>
<td>–</td>
<td>1.584</td>
<td>*</td>
<td>0.493</td>
<td>6.86</td>
<td>6.5 ± 0.3</td>
<td>6.2 ± 0.7</td>
<td>310.6</td>
</tr>
<tr>
<td>47 deg site</td>
<td>672-MS-black</td>
<td>47.1</td>
<td>2.721</td>
<td>4.8 ± 0.7</td>
<td>bd</td>
<td>1.05</td>
<td>9.4</td>
<td>15.2</td>
<td>270.6</td>
</tr>
<tr>
<td>Directly above M31, near M39, same site as 676-MS-white, in orifice</td>
<td>676-MS-yellow</td>
<td>41.2</td>
<td>2.096</td>
<td>0.0 ± 1.5</td>
<td>bd</td>
<td>4.02</td>
<td>6.4 ± 0.0</td>
<td>4.5 ± 0.4</td>
<td>456.6</td>
</tr>
<tr>
<td>Texture Garden (between M31 &amp; M39)</td>
<td>676-MS-black</td>
<td>30.8</td>
<td>3.032</td>
<td>3.3 ± 2.5</td>
<td>0.236</td>
<td>11.82</td>
<td>6.2 ± 0.4</td>
<td>4.8 ± 0.8</td>
<td>352.6</td>
</tr>
<tr>
<td>Hilo South area</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>M38</td>
<td>675-MS-white</td>
<td>43.3</td>
<td>2.408</td>
<td>9.2 ± 1.2</td>
<td>bd</td>
<td>2.91</td>
<td>5.2 ± 0.1</td>
<td>4.3 ± 0.2</td>
<td>432.6</td>
</tr>
<tr>
<td>M38</td>
<td>675-MS-yellow</td>
<td>42.4</td>
<td>2.647</td>
<td>9.6 ± 1.1</td>
<td>bd</td>
<td>2.67</td>
<td>5.9 ± 0.6</td>
<td>8.7 ± 1.8</td>
<td>522.6</td>
</tr>
<tr>
<td>M34</td>
<td>675-MS-black</td>
<td>47.4</td>
<td>1.925</td>
<td>12.0 ± 1.9</td>
<td>0.215</td>
<td>4.53</td>
<td>–</td>
<td>–</td>
<td>700.6</td>
</tr>
<tr>
<td>M34</td>
<td>675-MS-yellow2</td>
<td>48.2</td>
<td>2.660</td>
<td>–</td>
<td>bd</td>
<td>1.38</td>
<td>9.8</td>
<td>18.0</td>
<td>488.6</td>
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<td>M34</td>
<td>675-MS-red</td>
<td>47.4</td>
<td>0.705</td>
<td>*</td>
<td>bd</td>
<td>25.21</td>
<td>6.0 ± 0.3</td>
<td>2.7 ± 0.7</td>
<td>256.6</td>
</tr>
<tr>
<td>M34</td>
<td>675-MS-white2</td>
<td>48.1</td>
<td>2.508</td>
<td>4.8 ± 1.0</td>
<td>bd</td>
<td>1.16</td>
<td>11.4</td>
<td>20.2</td>
<td>450.6</td>
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<td>Pohaku area</td>
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<td></td>
<td></td>
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<tr>
<td>M57</td>
<td>671-MS-white</td>
<td>25.9</td>
<td>4.211</td>
<td>*</td>
<td>0.333</td>
<td>17.70</td>
<td>6.1 ± 0.6</td>
<td>3.2 ± 1.2</td>
<td>160.6</td>
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<tr>
<td>Ula Nui area</td>
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<tr>
<td>Ula Nui Mat</td>
<td>477-MS-blue</td>
<td>2.8</td>
<td>1.555</td>
<td>–</td>
<td>–</td>
<td>25.27</td>
<td>5.0 ± 0.3</td>
<td>2.1 ± 0.4</td>
<td>–</td>
</tr>
<tr>
<td>Background seawater</td>
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</tr>
<tr>
<td>M57 SW</td>
<td>476-niskin</td>
<td>5</td>
<td>0.015</td>
<td>–</td>
<td>–</td>
<td>42.61</td>
<td>6.2 ± 0.5</td>
<td>3.5 ± 0.5</td>
<td>–</td>
</tr>
<tr>
<td>Ula Nui SW</td>
<td>477-niskin</td>
<td>2.6</td>
<td>0.560</td>
<td>–</td>
<td>–</td>
<td>36.31</td>
<td>5.0 ± 0.1</td>
<td>2.9 ± 0.4</td>
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</tr>
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<td>Water column profiles</td>
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<td>Pele's Pit CTD cast</td>
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<td>0901-16</td>
<td>4.0</td>
<td>1.200</td>
<td>–</td>
<td>–</td>
<td>36.45</td>
<td>7.3 ± 0.3</td>
<td>5.3 ± 0.3</td>
<td>36.5</td>
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<tr>
<td>Pele's Pit, 2009</td>
<td>0901-14</td>
<td>3.7</td>
<td>0.690</td>
<td>–</td>
<td>–</td>
<td>42.76</td>
<td>6.9 ± 0.2</td>
<td>3.8 ± 0.1</td>
<td>42.8</td>
</tr>
<tr>
<td>Tow-yo west of summit</td>
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<tr>
<td>SW of Loihi</td>
<td>0904-02</td>
<td>3.7</td>
<td>0.286</td>
<td>–</td>
<td>–</td>
<td>41.79</td>
<td>6.2 ± 0.7</td>
<td>2.8 ± 0.2</td>
<td>108.8</td>
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<tr>
<td>SW of Loihi</td>
<td>0904-11</td>
<td>3.6</td>
<td>0.324</td>
<td>–</td>
<td>–</td>
<td>28.44</td>
<td>6.5 ± 0.7</td>
<td>3.6 ± 0.1</td>
<td>85.8</td>
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<tr>
<td>SW of Loihi</td>
<td>0904-15</td>
<td>3.7</td>
<td>0.167</td>
<td>–</td>
<td>–</td>
<td>41.41</td>
<td>6.5 ± 0.5</td>
<td>3.9 ± 0.7</td>
<td>95.8</td>
</tr>
<tr>
<td>SW of Loihi</td>
<td>0904-22</td>
<td>3.4</td>
<td>0.213</td>
<td>–</td>
<td>–</td>
<td>41.60</td>
<td>8.0 ± 0.2</td>
<td>6.0 ± 0.3</td>
<td>120.8</td>
</tr>
<tr>
<td>Pit of death CTD cast</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pit of death, 2009</td>
<td>0905-19</td>
<td>3.9</td>
<td>0.076</td>
<td>–</td>
<td>–</td>
<td>47.22</td>
<td>7.8 ± 0.4</td>
<td>4.9 ± 0.3</td>
<td>101.8</td>
</tr>
<tr>
<td>Pit of death, 2009</td>
<td>0905-01</td>
<td>3.7</td>
<td>1.500</td>
<td>–</td>
<td>–</td>
<td>45.28</td>
<td>6.3 ± 0.5</td>
<td>4.1 ± 0.1</td>
<td>105.8</td>
</tr>
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</table>
tunguishable from background seawater, with $\delta^{15}N_{\text{NH}_4}$ values ranging from +5.7 to +6.4‰ (mean = +6.0 ± 0.2‰) and $\delta^{18}O_{\text{NO}_3}$ values ranging from +2.6 to +3.6‰ (mean = +3.1 ± 0.3‰). The two samples collected at Ula Nui (4984 m) were distinctly different from those collected from Pele’s Pit, having lower $\delta^{15}N_{\text{NO}_3}$ and $\delta^{18}O_{\text{NO}_3}$ values of +5.0‰ and 2.4‰, respectively.

Nitrogen isotopes of ammonium were measured on a subset of hydrothermal fluid samples (Table 6). Because NH$_4^+$ isotopic composition is calculated by mass balance, we only report samples in which the ratio of NH$_4^+$ to the total inorganic N pool was at least 20% to minimize error propagation. $\delta^{15}N_{\text{NH}_4}$ values range from 0.0‰ to +16.7‰, with no observed correlation to NH$_4^+$ concentration or temperature across the sampling sites (not shown). Notably, the majority of $\delta^{15}N_{\text{NH}_4}$ values were near seawater NO$_3^-$ values or higher, with only two values exhibiting lower values of 0.0‰ and +3.3‰.

### 3.3. Energy availability

The amount of energy available from the 17 reactions listed in Table 3 were calculated for hydrothermal fluids that are characteristic of three locations in Pele’s Pit, Hiolo South, Pohaku and Hiolo North, and for three depths in a microbial mat sampled at the Ula Nui site (see Table 4 for compositions). Because most of the reactions shown in Table 3 yield a very small amount of energy, only the six most exergonic reactions are shown in Fig. 5. The amount of energy available from each of the reactions varies at each site (note that the scales in panels A and B in Fig. 5 are not the same). Under low energy conditions, Fig. 5A, iron oxidation coupled to nitrate reduction are among the most energy-dense reactions at Pohaku and in the top two parts of the Ula Nui mat. For the other sites under low-energy conditions, sulfide oxidation coupled to nitrate reduction reaction has the highest potential for microbial catabolism. For the high energy scenario, sulfide oxidation by nitrate has more potential than Fe oxidation at the Hiolo sites, while iron oxidation coupled to nitrate reduction has more potential to fuel microorganisms at the remaining sites.

Fe$^{2+}$, H$_2$S and NH$_4^+$ are the most significant electron donors in this environment, and NO$_3^-$ is the oxidant that yields the most energy. Reactions in which CH$_4$ is the electron donor and nitrite the electron acceptor yield so little energy that they would not be visible in Fig. 5. Fluids sampled at Pohaku have the greatest potential for nitrogen-based catabolic activities under the low energy scenario, but rank third behind the Hiolo sites under the high energy scenario. The broad concentration ranges of electron donors and acceptors at the Hiolo sites result in these two sites having the highest and lowest energy densities in the high and low energy scenarios, respectively.

Of the six reactions presented in Fig. 5, three are described as Fe$^{2+}$ oxidation by NO$_3^-$ (reactions (1)–(3) in Table 3). These reactions only differ with respect to the oxidation state of the product nitrogen species: NO$_2^-$, N$_2$ and NH$_3$. At all six sample sites, the Fe$^{2+}$ + NO$_3^-$ reaction to N$_2$ (Reaction (2)) is the most energy yielding of these reactions. Similarly, for the H$_2$S + NO$_3^-$ reactions (Reactions (11)–(14) in Table 3), the reaction in which N$_2$ (Reaction (11)) is the product species the most energy yielding of the sulfide oxidation reactions. N$_2$ was not measured during this work, but is inferred to be created in the subsurface as the deficit between the concentrations of NO$_3^-$ and NH$_4^+$ in the background seawater and that in the vent fluids, which is likely tens of μM.

### 3.4. Microbial diversity

Background seawater samples collected at 1100 m and 1700 m are comprised largely of Alpha-, Delta- and Gammaproteobacteria (Fig. 6). The Alphaproteobacterial orders Rhodobacterales, Rhodospirillales and the SAR11 group within the order Rickettsiales are abundant in these seawater samples, as are the SAR324 clade of Deltaproteobacteria and the Gammaproteobacterial orders Alteromonadales and Oceanospirillales. Archaeal communities in the background samples are comprised largely of Thaumarchaeota and Thermoplasmata.

Bacterial OTUs detected in Loihi fluids derive from 13 phyla and all 6 classes of Proteobacteria. OTUs classified

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**Fig. 4.** $\delta^{15}$N and $\delta^{18}$O isotopic ratios in NO$_3^-$ in Loihi fluids. Plot of $\delta^{15}$N-NO$_3^-$ and $\delta^{18}$O-NO$_3^-$ versus concentrations of NO$_3^-$ (A) and $\delta^{15}$N-NO$_3^-$ versus $\delta^{18}$O-NO$_3^-$ (B).
as Deltaproteobacteria in the order Syntrophobacterales are found in all three vent fluid samples from Pele's Pit, but not the sample LoihiPP6, collected at the Ula Nui site in 5000 m water depth. OTUs belonging to the order Thiotrichales within the γ-proteobacteria are abundant in LoihiPP2 (8.9%) and LoihiPP5 (5.4%), as are the OTUs within the Epsilon- (10.2% and 4.4%, respectively) and Zetaproteobacteria (13.1 and 10.1%, respectively) classes and the family Nitrospiraceae (18.9 and 19.2%, respectively). The genus *Thiohalophilus* is found in all three samples from Pele’s Pit at relative abundances of 1.7–6.6% but represents only 0.11% of the pyrotags from Ula Nui. Finally, sequences belonging to the SAR406 clade within the Deferribacteres are common to all four diffuse flow samples (0.83–12.8%), and the thermophilic, anaerobic genus *Caldithrix* is common to the three samples from Pele’s Pit (1.0–7.3%).

Archaeal OTUs common to Loihi subsurface fluids include the family Archaeoglobaceae (12.4–62.6% of all archaeal pyrotags), abundant in all three samples from Pele’s Pit, and Marine Benthic Group E in the Thermoplasmata, which was common in all four samples (8.3–36.1%). The Halobacteria present in the Pele’s Pit samples all derive from the order Halobacterales and either could not be classified further or belong to the Deep Sea Euryarcheotic Group. In LoihiPP1, Methanococci and M. menthanomicrobia are present (3.4 and 12.3%, respectively), but these are absent from the other samples.

Among the prokaryotic OTUs detected in venting fluids, a portion of them belong to groups known to participate in nitrogen redox cycling. These are largely grouped into NO₃⁻ reduction/denitrification, N-fixation and NO₂⁻ oxidation (Fig. 6). Among these, the most abundant putative N-fixing microbes include *Caldithrix*, from which some members perform dissimilatory nitrate reduction to ammonium, or DNRA (Miroshnichenko et al., 2003), Epsilon-proteobacteria, *Thiohalophilus* and members of the SAR324 clade. Putative N-fixers detected include members of the bacterial order Chlorobiales and archaeal methanogens in the genera *Methanococcus* and *Methanothermococcus*. Members of the phylum Nitrospirae are present in all four samples, and are abundant in LoihiPP2 and LoihiPP5. Approximately 4 and 10% of the sequences were assigned to the genus Thermodesulfovibrio in LoihiPP2 and LoihiPP5, respectively, while only a few sequences, <1% in LoihiPP2 and none in LoihiPP5, were assigned to the genus *Nitrospira*. The majority of sequences classified as Nitrospirae could not be classified beyond Nitrospiraceae, therefore it is impossible to guess their role in N-cycling given that some members of this family are nitrite oxidizers (*Nitrospira*) while others are not (Thermodesulfovibrio and others).

It should be noted that OTUs from the genera *Mariobacter* and *Halomonas* and the NO₂ oxidizing genus *Nitrospina* were abundant in fluid samples, but the same OTUs were detected in abundance in the background samples as well, and therefore do not appear in the background subtracted libraries reported (although different OTUs of *Mariobacter* not detected in the background samples are present). Both *Mariobacter* and *Halomonas* are cos-
Genes for nitrate reductase belonging to both genera have been detected in low temperature vent fluids and on active hydrothermal vent sulfides (Pérez-Rodríguez et al., 2009).
ical assimilation can also have an effect on 
ated dissimilatory N-redox cycling, discussed below, biolog-
subsurface environment. In addition to biologically medi-
Childress, 1994 ).

Neubeck, 2009) and therefore could be favorable in Loihi’s 
1993; Ottley et al., 1997; Smirnov et al., 2008; Holm and 
measured NO2/C176 

terests is not surprising. Given the abundance of Fe2+ in 
these fluids, the mixing zone where subsurface fluids meet 
the seafloor likely represents a kinetic battleground between 
Fe-oxide precipitation and microbial utilization of oxygen 
for oxidation of compounds including NH4 and NO2. In 
order to shed more light on the nature of N-cycling reac-
tions occurring, we also examined the N and O stable iso-
opic composition of N-bearing species. To our 
knowledge, only one study has reported on coupled N 
and O stable isotope measurements in the context of bio-
geochemical cycling of nitrogen species in a deep-sea 
hydrothermal system (Bourbonnais et al., 2012a). Using 
samples from the Endeavour Segment and Axial Volcano 
on the Juan de Fuca Ridge, these authors found evidence 
for removal of NO3 from fluids primarily by dissimilatory 
processes when NH4 concentrations were ≤10 μM, condi-
tions representative of their diffuse flow sites as well as at 
those sampled at Loihi. Indeed, in a related study using 
15N isotope labeling, Bourbonnais et al. (2012a) observed 
the highest rates of nitrogen removal from these same sites, 
confirming the importance of reductive nitrate consump-
tion. In addition to evidence for cycling involving NO3, 
Bourbonnais et al. (2012a) also found evidence for both 
consumption and production of NH4 by microbial activity. 
This important initial work indicated that microbial denitri-
fication is a primary route of inorganic nitrogen loss in dif-
use fluids, but also noted possible spatial and temporal 
heterogeneity in N redox processes. However, the sites on 
the Juan de Fuca Ridge and Axial Seamount exhibit high 
concentrations of sulfide, which strongly influence the com-
position of the resident microbial communities. In contrast, 
fluids from Loihi Seamount, with low sulfide and high iron, 
represent a starkly different geochemical context for low-
temperature venting.

Hydrothermal fluids having NO3 concentrations lower 
than background seawater can stem from either abiotic 
or biological consumption of NO3, as mentioned above, 
or from dilution of fluids containing little or no NO3. 
While dilution would have no influence on isotopic com-
position, isootope fractionation by biological reduction of 
nitrate leads to increases in both δ15N and δ18O of the 
remaining nitrate pool (Granger et al., 2008), allowing 
one to discern between biological consumption and phys-
ical mixing processes. Indeed, nitrate reduction, whether 
by dissimilatory or assimilatory processes, has been 
shown to impart distinctly parallel (e.g. equal) isotope 
effects for both N and O, leading to a characteristic 1:1 
dual isotopic evolution (e.g., slope of 1 in Fig. 4). The 
elevated N and O isotope ratios of NO3 in the 
hydrothermal fluids of Pele’s Pit clearly reflect the influ-
ence of biological NO3 consumption. However, in con-
trast to the parallel 1:1 increase in δ15NO3 and 
δ18ONO3 (relative to the composition of background sea-
water) expected from isotopic fractionation due to NO3 
consumption alone, changes in the δ18ONO3 values are

4. DISCUSSION

4.1. Biogeochemistry and isotope systematics at Loihi

While hydrothermally sourced Fe and CH4 have been 
recognized as important energy sources for microbial meta-
obolism at Loihi (Gamo et al., 1987; Emerson and Moyer, 
2002), the role of N-redox transformations in supporting 
subsurface microbiologically mediated N-cycling is much less 
understood, in part due to the lack of measurements of 
inorganic N species at Loihi since the first studies that took 
place two decades ago (Karl et al., 1989; Sedwick et al., 
1992). Those early studies of Loihi revealed elevated NH4 
in Loihi hydrothermal fluids in samples collected from 
Pele’s vents prior to the July–August 1996 seismic events 
that resulted in the collapse of Pele’s vents and the creation 
of the pit crater Pele’s Pit (Hilton et al., 1998). Immediately 
following the creation of Pele’s Pit, venting hydrothermal 
fluid temperatures reached 200 °C (Wheat et al., 2000), 
followed by a slow decrease in temperatures during 1997–1999 
(Malahoff et al., 2006). Sampling of Loihi vents during 
2006–2008 revealed that end-member fluid temperatures 
were 21–55 °C, similar to pre-1996 values (Glazer and 
Rouxel, 2009), and that Fe/Mn ratios returned to ~30, 
the same as pre-1996 values (Glazer and Rouxel, 2009), 
indicative of a return to a steady state resembling pre-
ruption conditions. Our hydrothermal fluid NH4 data is 
similar in range to that of the earlier work (Fig. 3) and is 
agree in agreement with a return to steady state at Loihi. We also 
measured NO3 concentrations at Loihi for the first time. 
Concentrations were below detection for half of the samples 
collected and ~0.10–0.50 μM for the rest. Although low, 
these levels of NO3 are consistent with active redox cycling 
involving NO3 as a product of NH4 oxidation and/or NO3 
reduction, both reactions that are favorable under in situ 
conditions (Fig. 5).

Concentrations of NO3 + NO2 and NH4 in Loihi fluids 
are strongly negatively correlated (Fig. 3, Table 5), suggest-
ing linkages between the redox cycling of these inorganic 
nitrogen species. These linkages may be the result of simulta-

cous abiotic and biotic mechanisms in Loihi’s subsur-
face, with neither possibility being mutually exclusive. 
NO3 can be reduced abiotically to NH4 with Fe2+ as a cata-
lyst between 22 °C and 200 °C (Summers and Chang, 
1993; Ottley et al., 1997; Smirnov et al., 2008; Holm and 
Neubeck, 2009) and therefore could be favorable in Loihi’s 
subsurface environment. In addition to biologically medi-
dated dissimilatory N-redox cycling, discussed below, biolog-
ical assimilation can also have an effect on N-isotope 
composition in hydrothermal environments (Lee and 
Childress, 1994).

Unlike NH4, NO3 shows no correlation to NO3 + NO2 
or dSi. The lack of correlation with dSi suggests that it is of 
low-temperature origin, likely released as a reactive inter-
mediate of a biological process (i.e., not an endmember pro-
duct of high temperature reactions). As NO3 is an 
intermediate of both denitrification and nitrification, the 
lack of correlation with conservative and non-conserved 
tracers is not surprising.
much larger than changes in $\delta^{15}N$ values, suggesting that processes other than NO$_3^-$ reduction are also occurring. Indeed, such deviations from a 1:1 covariation in dual isotope space for NO$_3^-$ have been observed in other marine systems including oxygen minimum zones (Sigman et al., 2005; Casciotti and McIlvin, 2007; Bourbonnais et al., 2012a), shallow surface water environments (e.g., Wankel et al., 2007) and even other deep biosphere environments (Wankel et al., 2015), and have been interpreted as reflecting the combined effects of NO$_3^-$ consumption (via reduction) and NO$_3^-$ regeneration (via nitrification). Results of a recent modeling study suggest that isotopic signatures of nitrification evident in denitrifying systems might be a universal characteristic of nitrogen cycling in aquatic systems (Granger and Wankel, 2016).

Given the prevalence of NH$_4^+$ in the hydrothermal fluids at Loihi, we suggest that the contribution of (1) partial NH$_4^+$ oxidation and (2) possibly rapid NO$_2^-$ reoxidation leads to the observed deviation of NO$_3^-$ dual isotope composition from the 1:1 line (Fig. 4). This dynamic arises because N and O isotope enrichments in NO$_3^-$ are tightly coupled during consumption (e.g., Granger et al., 2008), while the production of NO$_3^-$ by nitrification (both ammonia oxidation to nitrite, as well as nitrite oxidation to nitrate) represents a unique decoupling of these two isotope systems as discussed further below (Casciotti and McIlvin, 2007; Wankel et al., 2007; Sigman et al., 2009). Foremost, under the mesophilic conditions at the Loihi vents, the partial oxidation of the NH$_4^+$ pool by ammonia oxidizing microbes, which is known to have a large N isotope effect (14–38‰; Casciotti et al., 2003; Santoro and Casciotti, 2011), would result in production of low $\delta^{15}N_{NO_3}$. Indeed, the occurrence of elevated $\delta^{15}N_{NH_4}$ values in Loihi fluids (up to +16‰), strongly supports that oxidative processes have partially consumed the vent derived NH$_4^+$ pool. While it is impossible to accurately estimate the $\delta^{15}N_{NO_3}$ of newly produced NO$_3^-$ from a partially oxidized NH$_4^+$ pool using the existing data (i.e., it is difficult to estimate the fraction of NH$_4^+$ consumed at these low concentrations and the isotope effects for NH$_4^+$ oxidation range quite widely (Casciotti et al., 2003)), it is clear that the contribution of this newly produced NO$_3^-$ having a very low $\delta^{15}N$ value would act to shift the bulk NO$_3^-$ dual isotopic composition to the left of the 1:1 line evolving from a background seawater source (Fig. 4).

The oxygen isotope composition of newly produced NO$_3^-$ may also play a role in the observed deviation from the 1:1 line, specifically implicating nitrite oxidation (and nitrite oxidizing bacteria). The source O atoms of new NO$_3^-$ originate from both H$_2$O and O$_2$ (Buchwald and Casciotti, 2010; Casciotti et al., 2010) with kinetic isotope effects at each step of O atom incorporation as well as the potential for oxygen isotope equilibration between the NO$_2^-$ intermediate pool and water (Casciotti and McIlvin, 2007; Buchwald and Casciotti, 2013). In general, it is believed that the combination of these influences results in the $\delta^{18}O$ of newly produced NO$_3^-$ to be near $+1.9 \pm 3\%e$ in seawater (Buchwald et al., 2012). Given the low pH of the Loihi fluids, $\sim$5.7–6.5 (Glazer and Rouxel, 2009), it is safe to assume that the $\delta^{18}O$ of the intermediate nitrite pool (whether derived from NH$_4^+$ oxidation or NO$_2^-$ reduction) is in isotopic equilibrium with the ambient water – which would yield a value of $\sim$14‰ (Casciotti and McIlvin, 2007). During partial oxidation of this NO$_2^-$ pool, the kinetic isotope effects associated with both NO$_2^-$ oxidation ($\delta^{18}O_{NO_2}$) as well as incorporation of an O atom from H$_2$O ($\delta^{18}O_{H_2O}$), would culminate in production of new NO$_3^-$ with a $\delta^{18}O$ value of between +4 and +12‰ (see Buchwald and Casciotti, 2010), higher than that of background seawater. In support of this mechanism, our data reveal the presence of known nitrite-oxidizing genera in the family Nitrospiraceae. As indicated in Fig. 6, the combination of NO$_3^-$ reduction by denitrifying microbes together with nitrification (both the partial oxidation of the NH$_4^+$ pool as well as the reoxidation of NO$_3^-$) act in opposing directions, modulating the evolving NO$_3^-$ dual isotopic composition to fall above the 1:1 line predicted by denitrification alone. Co-occurring denitrification and nitrification was found to occur in Beggiatoa mats in Guaymas Basin (Winkel et al., 2014), indicating this may be a widespread feature in hydrothermal systems hosting sharp gradients of oxygen and nitrogen species. In summary, our data clearly suggest that both microbially mediated reductive and oxidative processes play a joint role in regulating fluxes of dissolved inorganic nitrogen from the Loihi subsurface. Although hydrothermal vent N isotope data is sparse, such NO$_3^-$ dual isotope dynamics have also been recently observed in other hydrothermal systems (Bourbonnais et al., 2012a), reflecting the simultaneous influence of a range of redox reactions at a sharp fluid-mixing zone. Importantly, the data from Loihi reveals that this range of redox reactions also occurs in a hydrothermal system with low concentrations of dissolved H$_2$S and high concentrations of dissolved Fe$^{2+}$. This indicates that the presence or absence of H$_2$S and metabolisms coupling H$_2$S and N-redox transformations do not greatly alter the N-isotope systematics in diffuse flow hydrothermal vent environments. The precise cause requires more study, but may reflect substitution of N-redox processes coupled with H$_2$S oxidation with other oxidative processes (Fe$^{2+}$ oxidation, for example), that H$_2$S is more important in the subsurface biosphere at Loihi but not abundant as measured in samples collected at the seafloor, or that H$_2$S is not a strong influence on N-redox processes.

We note also that the NO$_2^-$ dual isotope values from Ula Nui are slightly lower in $\delta^{15}N$ and $\delta^{18}O$ than background waters near the Pele’s Pit crater and look more similar to background seawater than vent fluids. A likely scenario explaining these data is that the water in the matrix of the mats at the Ula Nui site is derived more from deep seawater than the ultra diffuse fluids emanating from the seafloor at that site (Edwards et al., 2011).

4.2. Energetics from N-redox reactions in the Loihi subsurface

Microorganisms are known to catalyze nitrogen redox reactions in order to gain energy (see Amend and Shock
could be supported on a typical maintenance level (e.g.,
led into biomass synthesis, then between 1 liter of hydrothermal fluid. If 0.1 J (kg H₂O)
be available for microbial processes varies considerably. Most studies that present energetic analyses of potential microbial metabolisms in units of energy densities do so because they are quantifying the disequilibrium resulting from the mixing of seawater with hydrothermal fluids (McCollom and Shock, 1997; Amend et al., 2011). Because the composition of hydrothermal fluids can vary dramatically depending upon the types of rocks that the hydrothermal fluids circulate through, the resulting amount of redox energy that can be available for microbial processes varies considerably. For instance, fluids from ultramafic hydrothermal systems that mix with seawater can provide up to 3700 J (kg H₂O)⁻¹ for H₂ oxidation with O₂ as the electron acceptor (McCollom, 2007), while seawater mixing with basalt-derived fluids at a mid-ocean ridge system (East Pacific Rise, EPR, 21°N OBS vent) only makes about ~35 J (kg H₂O)⁻¹ available for the same reaction (Shock and Holland, 2004). On the other end of the spectrum, potential energy yields for some reactions due to fluid mixing can be less than 10⁻⁴ J (kg H₂O)⁻¹ (Price et al., 2015). The larger values noted above are likely outliers for most natural systems since they are capturing the mixing of two radically distinct fluids instantaneously. In environmental settings that are not subjected to such dramatic gradients, the energy densities are on par or smaller than those shown in Fig. 5. (LaRowe et al., 2014; Osburn et al., 2014; Teske et al., 2014; Price et al., 2015).

All of the reactions whose energy densities are shown in Fig. 5 supply more than 0.1 J (kg H₂O)⁻¹. Although this may not seem like a large amount of energy, it is worth noting that maintenance energies for microorganisms range from 0.019 to 4700 × 10⁻¹² J (s cell)⁻¹ (LaRowe and Amend, 2015). This means that a community of 10⁶ cells could be supported on a typical maintenance level (e.g., 10⁻¹⁴ J (s cell)⁻¹) by any of the reactions considered at Loihi for almost 4 months using only the constituents of 1 liter of hydrothermal fluid. If 0.1 J (kg H₂O)⁻¹ were channeled into biomass synthesis, then between ~10⁷ and 10⁹ cells could be produced, depending on the sources of C, N, S, the overall redox state and other physiochemical variables (LaRowe and Amend, 2016).

4.3. Microbial diversity in the Loihi subsurface

The temperatures of hydrothermal fluids at Loihi make it comparable to diffuse-flow hydrothermal sites at spreading centers and seamounts. However, unlike the majority of these systems, sulfide concentrations are only moderately elevated relative to background seawater at Loihi (Sedwick et al., 1992). Thus, perhaps not surprisingly, sulfur oxidizing Epsilonproteobacteria represent only 0.15–10.3% of the bacterial communities in the four subsurface fluid samples analyzed here (Fig. 6). In contrast, previous studies of diffuse hydrothermal fluids with high concentrations of H₂S found that Epsilonproteobacteria represented a large proportion of the total bacterial community (Huber et al., 2007, 2010; Bourbonnais et al., 2012b). For example, in fourteen samples of diffuse fluids venting at five different seamounts along the Mariana Arc, with one exception, Epsilonproteobacteria comprised 15–87% of the total bacterial community, with a mean value of 37.4% (Huber et al., 2010). At Axial volcano, on the Juan de Fuca Ridge, Epsilonproteobacteria comprise up to 80% of the total bacterial community (Huber et al., 2007; Bourbonnais et al., 2012b). In those studies, the major genera of Epsilonproteobacteria detected at each vent site were variable, but members of Sulfurimonas, Sulfurovum and Hydrogenomonas were predominant. Sulfurimonas, Sulfurovum, Hydrogenomonas and Nitratiruptor combined comprised >99% of the Epsilonproteobacteria sequences detected in the Loihi samples. Fluids from the area where LoihiPP2 and LoihiPP5 were collected were ~50°C, and contained little to no O₂ (below detection, or <3 μM) and ~2–4 μM HS⁻ during the time of sampling (Glazer and Rouxel, 2009). These conditions are ideal for the Epsilonproteobacteria detected, while reduced sulfur compounds were below detection at the sites where they were not detected, Marker 34 and Ula Nui (Glazer and Rouxel, 2009; Edwards et al., 2011). Cultured representatives from all the Epsilonproteobacterial genera detected here are NO₃ reducers with the conserved periplasmic nitrate reductase (nap) gene pathway for this process (Vetriani et al., 2014), suggesting their importance in NO₃ reduction at Loihi. Despite their lower abundance than at other vent sites, Epsilonproteobacteria still represent the most abundant putative NO₃ reducers. In addition to the Epsilonproteobacteria detected, other detected NO₃ reducers or denitrifiers include Gammaproteobacteria in the genera Thiohalophilus, Marinobacter and Halomonas as well as the genus Caldithrix. While Gammaproteobacteria from the SUP05 clade were noted as abundant denitrifiers at Axial Volcano (Bourbonnais et al., 2012b), they were not detected at Loihi, likely due to the low abundance of H₂S. A related study detected heme-containing nitrite reductase (nirS) genes related to Pseudomonas spp. in diffuse flow hydrothermal vent fluids along the Endeavour Segment (Bourbonnais et al., 2014), but Pseudomonas were also not detected at abundances >0.3% in our hydrothermal fluids samples. Pseudomonas was detected in the background samples at abundances of
work of Bourbonnais and colleagues on the Juan de Fuca simultaneously. A similar conclusion was drawn from the oxidative and reductive processes are likely occurring logically mediated in Loihi subsurface fluids, and that both data that N-cycling processes are occurring and likely bio-
measurements, isotope systematics, energetic calculations (Haroon et al., 2013; Offre et al., 2013), this is still a rela-
Archaea are known to participate in denitrification (Swingley et al., 2012). This
possess genes in the V6 region of SSU rRNA to confidently assign the
Halobacteria and Archaeoglobi were both abundant in Loihi fluids, there is not enough phylogenetic resolution in the V6 region of SSU rRNA to confidently assign the sequences recovered to one of the denitrifying genera. It is possible that members of the Thermoproteales are participating in denitrification at Loihi, although they were present in low abundances here. Recent metagenomic analysis revealed that members of the Thermoproteales possess genes in the nir and nar pathways, indicative of NO3− and NO2− reduction (Swiningle et al., 2012). This group was present at 0.05, 0.22 and 0.20% relative abundance in the archaeal pyrotag libraries from Loihi PP1, PP2 and PP5, respectively, indicating a potential additional role for archaeal denitrification at Loihi by these organisms. Putative N-fixing Bacteria and Archaea were detected in the Hiolo North area, although representing only a minor percentage of the entire population (Fig. 6). While some Archaea are known to participate in denitrification (Haroon et al., 2013; Offre et al., 2013), this is still a relatively underexplored metabolic pathway in Archaea. N2 is likely abundant as indicated by the deficit between seawater NO3− + NO2− and the sum of measured N species in end-member fluids presented here, suggesting that N-fixation in low-temperature diffuse fluids at Loihi may be occurring. N-fixation in the warm Loihi subsurface environment is also suggested from two samples with δ15N values lower than background seawater NO3− (0.0% and +3.3%); rem-
inalization of biomass supported by N-fixing microbes would generate NH4+ having δ15N values near 0‰ (Delwiche and Steyn, 1970; Meador et al., 2007). It is also possible, however, that these values are indicative of low δ15N produced NH4+ from DNRA, which has been shown to have an isotope effect of −6 to −8‰ in hydrothermal vent isolates (Perez-Rodriguez et al., 2013b) and which would therefore generate NH4+ with a δ15N of between −2 and 0‰ from bottom seawater NO3− (δ15N ~+6‰).

4.4. Conclusions

The combined data presented here on biogeochemical measurements, isotope systematics, energetic calculations and microbial diversity present strong multidisciplinary data that N-cycling processes are occurring and likely biologically mediated in Loihi subsurface fluids, and that both oxidative and reductive processes are likely occurring simultaneously. A similar conclusion was drawn from the work of Bourbonnais and colleagues on the Juan de Fuca Ridge (Bourbonnais et al., 2012a,b), and cryptic N-cycling was explicitly demonstrated in Beggiatoa mats in Guaymas Basin, where Beggiatoa perform denitrification in concert with attached nitrifiers (Winkel et al., 2014). Thus, there is a growing consensus that subsurface N-cycling processes are linked and complicated, but the role of N-cycling in driving subsurface biogeochemistry and microbiology is still underexplored.

Like Loihi, there are many hydrothermal systems with elevated concentrations of Fe²⁺ and low concentrations of sulfide around the globe, including the Marianas back-arc (Davis and Moyer, 2008) and diffuse vents along the Mid-Atlantic Ridge (Scott et al., 2014). Therefore, the work presented here can be interpreted to potentially represent high Fe, low sulfide systems elsewhere. Additionally, our results are in agreement with those derived from the Juan de Fuca Ridge and Axial Volcano, where sulfide is abundant (Bourbonnais et al., 2012a,b), indicating that trends presented here are potentially representative of low-temperature venting systems in general, which represent up to 90% of venting worldwide (Elderfield and Schultz, 1996).

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