Supplemental File 2

In [1]:
import numpy as np
from numpy import ma
import matplotlib
import matplotlib.pyplot as plt
from matplotlib import cm

We hypothesize that preference for ammonium assimilation over nitrogen fixation consumed ammonium diffusing inwards into the ANME-SRB consortia shown in Figure 8, forcing cells in the consortium interior to fix additional nitrogen from $^{15}N_2$ to compensate for the decrease in N source from ammonium uptake. Here, we model ammonium diffusion into an ANME-SRB consortium and simultaneous ammonium assimilation as a classic 1D diffusion problem with boundary conditions describing continuous input at a fixed location ($x = 0$) with decay:

$$ \frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial t^2} - kC $$

where $C$ is the concentration of ammonium at time $t$, $D$ is the diffusivity for dissolved ammonium, and $k$ is the first-order rate constant for ammonium assimilation.

Solving this differential equation at steady state ($\frac{\partial C}{\partial t} = 0$) gives:

$$ C = C_0 \exp\left(-\sqrt{\frac{k}{D} x}\right) $$

where $C_0$ is the concentration of ammonium in the porewater and thus the concentration at the exterior of the consortium.

Re-arranging to solve for $x$ yields

$$ x = \frac{-\ln\left(\frac{C}{C_0}\right)}{\sqrt{\frac{k}{D}}} $$

In our approach, we explore $x(C_0, D)$ holding $C$ constant at 25 µM, the threshold value of ammonium concentration above which diazotrophy is inhibited in incubations of methane seep sediments (Dekas, et al., 2018 [https://sfamjournals.onlinelibrary.wiley.com/doi/full/10.1111/1462-2920.14342]). $x(C_0, D)$ will thus describe the depth within a consortium at which ammonium will be depleted sufficiently to induce diazotrophy, described in the figure below as ‘diazotrophy frontier depth’. We have calculated $k = 0.0004$-$0.0009$ hr$^{-1}$ from time-series measurements of ammonium concentration in methane seep sediment incubations under ammonium-replete ($\geq$ 25 µM) conditions (Dekas, et al. 2018, Fig. S5, "Mat-774", "Mat-794").
We explore a range of $C_0$ between 0.01 and 316 µM, representative of porewater ammonium concentrations measured in situ (Dekas, et al. 2018).

In [2]:
```
C = 25
k = 0.0004
```

We use an estimate for the diffusivity of ammonium derived from the literature (Krom and Berner, 1980 (https://aslopubs.onlinelibrary.wiley.com/doi/pdf/10.4319/lo.1980.25.2.0327)) of $D = 3.5 \times 10^6$ to constrain maximum possible diffusivity, and explore a range of parameter values for $D$ down to $10^1$, representing the limitations on diffusion imposed by diffusion between cells within the consortium. A large range of values for $D$ was employed here to reflect the challenges of measuring this parameter within ANME-SRB consortia.

In [3]:
```
C_0 = np.logspace(-2, 2.5, 1000)
```

In [4]:
```
D = np.logspace(1, 6.7, 1000)
```

In [5]:
```
c_0, d = np.meshgrid(C_0, D)
x = np.zeros((1000, 1000))
x = -np.log(25/c_0) * 1/np.sqrt(k/d)
```
In [7]:

```python
fig, ax = plt.subplots()

x = ma.masked_where(x <= 0, x)
norm = cm.colors.LogNorm()
cs = ax.contourf(c_0, d, x, 50,
                 norm = norm)
cbar = fig.colorbar(cs)
plt.xlim(25, c_0.max())
plt.xlabel('Porewater [NH$_4^+$] (µM)')
ax.set_yscale('log')
ax.set_xscale('log')
ax.set_xticks([30, 50, 100, 300])
ax.get_xaxis().set_major_formatter(matplotlib.ticker.ScalarFormatter())
plt.ylabel('Diffusivity (µm$^2$ h$^{-1}$)')
cbar.set_label('Diazotrophy frontier depth (µm)')
plt.rcParams['figure.figsize'] = [10, 10]
plt.rcParams['font.size'] = 12
plt.show()
```
Plotting the results, we see that the depth at which we observe significant $^{15}N$ incorporation and thus diazotrophic activity in our consortia (1 to 10 $\mu$m into consortia, Fig. 8) is possible at diffusivities near that measured for bulk marine sediment ($\approx 10^6 \text{ } \mu m^2 \text{ } hr^{-1}$) for porewater ammonium concentrations that approach 25 $\mu$m. Thus, the simple model presented here broadly supports our hypothesized mechanism for the observed gradient in diazotrophic activity presented in Figure 8.