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 $\left(\frac{\partial C}{\partial t} = 0\right)$ 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 (≥ 25 μM) conditions (Dekas, et al. 2018, Fig. S5, "Mat-774", "Mat-794").

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

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

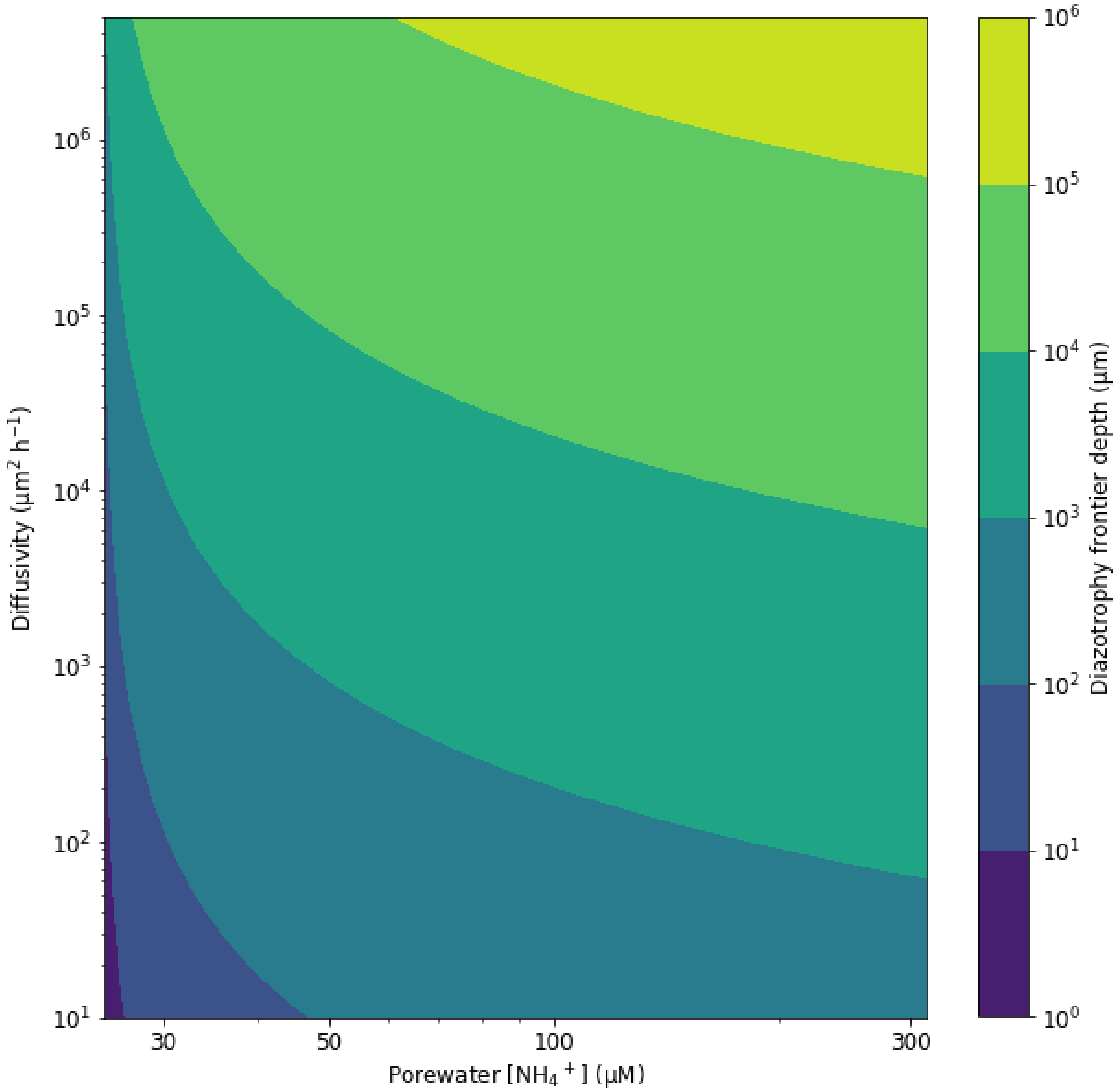
We use an estimate for the diffusivity of ammonium derived from the literature ([Krom and Berner, 1980 (https://aslopubs.onlinelibrary.wiley.com/doi/pdf/10.4319/lo.1980.25.2.0327)](https://aslopubs.onlinelibrary.wiley.com/doi/pdf/10.4319/lo.1980.25.2.0327)) of D = 3.5 * 10^6 to constrain maximum possible 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 [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]:
```
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$$^+$] ($\mu$M)')
ax.set_yscale('log')
ax.set_xscale('log')
ax.set_xticks([30, 50, 100, 300])
ax.get_xaxis().set_major_formatter(matplotlib.ticker.ScalarFormatter())
plt.ylabel('Diffusivity ($\mu$m$^2$ h$^{-1}$)')
cbar.set_label('Diazotrophy frontier depth ($\mu$m)')
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 μm into consortia, Fig. 8) is possible at diffusivities near that measured for bulk marine sediment (≈10^6 μm^2 hr^{-1}) for porewater ammonium concentrations that approach 25 μm. Thus, the simple model presented here broadly supports our hypothesized mechanism for the observed gradient in diazotrophic activity presented in Figure 8.