The Laurentian Great Lakes hold 20% of Earth's surface freshwater and provide essential ecosystem services. Moreover, as an interconnected waterway that spans strong environmental gradients, the Great Lakes represent a unique natural laboratory for understanding how physical, chemical, and biological forces interact to shape microbial communities and biogeochemistry. Here we explore the drivers of microbial diversity and activity across the Great Lakes, using samples collected as part of an ongoing multi-year time series. First we characterized community composition across lakes, depths, seasons, and years. We found that depth and light are strong drivers of community structure in stratified water columns. Across surface waters, we found distinct microbial signatures in each of the Great Lakes, reflecting their biogeochemical variability. To explore metabolic functions, we then sequenced 21 metagenomes collected from all five Great Lakes, totaling over 1 billion sequences. We used genome assembly and binning approaches to reconstruct 572 metagenome-assembled genomes (MAGs) and created a microbial tree of life for the Laurentian Great Lakes. We mapped ecological distribution patterns for these genomes and found distinct distributions for taxa and metabolisms across lakes and depths. We focus here on two important groups for ecology and biogeochemistry, the cyanobacteria and nitrifying Bacteria and Archaea. Our work represents the first picture of microbial diversity across the entire Laurentian Great Lakes and is an essential baseline from which to monitor future ecosystem change.