Sustainable transportation idea includes not only switching from conventional energy sources to alternative fuel resources, but also diverging from private vehicle use and shifting to alternative transportation modes. As a part of alternative transportation mode, utilizing alternative fuels in public transportation operation supports sustainable transportation at full glance. Given their implications in terms of air quality and sustainable movement of people, transit buses, which provide the primary public transportation service, are considered an ideal domain for the deployment of alternative fuels. An input-output (IO) model is developed based on Eora database a detailed IO database that consists of national IO tables. Using the Eora-based IO model, this study quantifies and assesses the environmental, economic, and social impacts of alternative fuel buses in Atlanta, GA, and Miami, FL based on 6 macro-level sustainability indicators. The life cycle sustainability performance of these buses are then compared to that of a diesel transit bus as well as a regional comparison is carried out based on the two U.S. metropolitan areas. Based on these results, a multi-objective optimization model is constructed to find an optimal transit bus fleet for the studied U.S. regions. It has been found that a transit fleet that is composed of diesel buses operating in Atlanta has 30% more global warming potential than that of a transit fleet operating in Miami. The same bus fleet operating in Atlanta incurs a life cycle cost (LCC) that is more than double the LCC of the fleet operating in Miami. The study presents a way in which transit agencies can strategize their efforts to transition to a sustainable bus fleet.