**Table 4** Comparison between mNGS and 16S rRNA sequencing

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|  | **mNGS** | **16S rRNA sequencing** |
| **Principles** | Total microbial DNA from environmental samples was mechanivally fragmented, ligated with universal adapters, PCR-amplified, and sequenced. Resulting short reads were then assembled into contgs for subsequent analysis | Amplicon sequencing targeting hypervariable regions (e.g., V3-V40 of the 16S rRNA gene, following PCR amplification with universal primers |
| **Scope** | This approach provids comprehensive coverage of microbial genomes within the sample, encompassing bacteria, fungi, viruses, and other microbial domains | mainly targeting bacteria and archaea |
| **Species identification** | Ability to identify microorganisms down to the species or even strain level | Often only identified to genus or species level, and in some cases with limited accuracy at species level |
| **Volume of data and complexity of analysis** | Large volume of data and complex bioinformatics analyses | Relatively small amount of data and simple to analyse |
| **Cost and time** | Higher cost and longer time | Lower cost and shorter time |
| **Fields of application** | Applicable for comprehensive profiling of microbial community composition and function, including environmental microbiology and clinical diagnostics | Routinely employed for composition and diversity analysis of microbial communities in fields such as microbial ecology and environmental science |