Rumen protozoa are anaerobic ciliates that degrade feedstuff consumed by the host. The objective of our study was to determine if breed (B) or lactation stage (LS) affect the rumen protozoal community. Whole rumen digesta samples were collected at 3 and 93 days in milk (DIM) from seven Holstein (H), eight Jersey (J), and seven Holstein-Jersey crossbreeds (X) co-housed in free stalls. To characterize the rumen protozoa, the V3-V4 region of the 18S rRNA gene was amplified. Illumina MiSeq (v.3) produced sequence reads and the program Mothur was used for all bioinformatic analyses. The protozoal densities (cells/mL digesta) were determined by real-time PCR amplification of the 18S rRNA gene. The effects of B, LS, or B x LS on rumen protozoa were statistically evaluated with the PROC MIXED model in SAS. At 3 DIM, 307,739 sequence reads clustered at a 4% cutoff into 3-21 operational taxonomic units (OTU) per animal with means of 5, 6, and 7 for H, J, and LS, respectively. At 93 DIM, 536,846 sequence reads clustered into 1-15 OTU per animal with an average of 3 OTU for each breed. For both stages, greater than 98% of the sequences clustered with OTU 1, belonging to the family, Ophryoscolecidae. The OTU-based diversity measures, Shannon index (P<0.01), Inverse Simpson index (P<0.05), and Chao I estimator (P<0.001) showed greater diversities at 3 DIM. The abundances of protozoal genera Epidinium (0.4-9.3% abundance), Ostracodinium (3.5-21.6%), Ophryoscolex (0.2-3.0%), and Polyplastron (2.4-7.1%) were affected by LS (P<0.05). Eudiplodinium spp were less abundant in H than in X (P<0.05), respectively, at 3 DIM (H: 2.0, X: 12.2%) and at 93 DIM (H: 2.6, X: 20.3%). Protozoal densities did not differ. The present study suggests that early and peak LS, but not B, impacts the rumen protozoal community in primiparous dairy cattle.