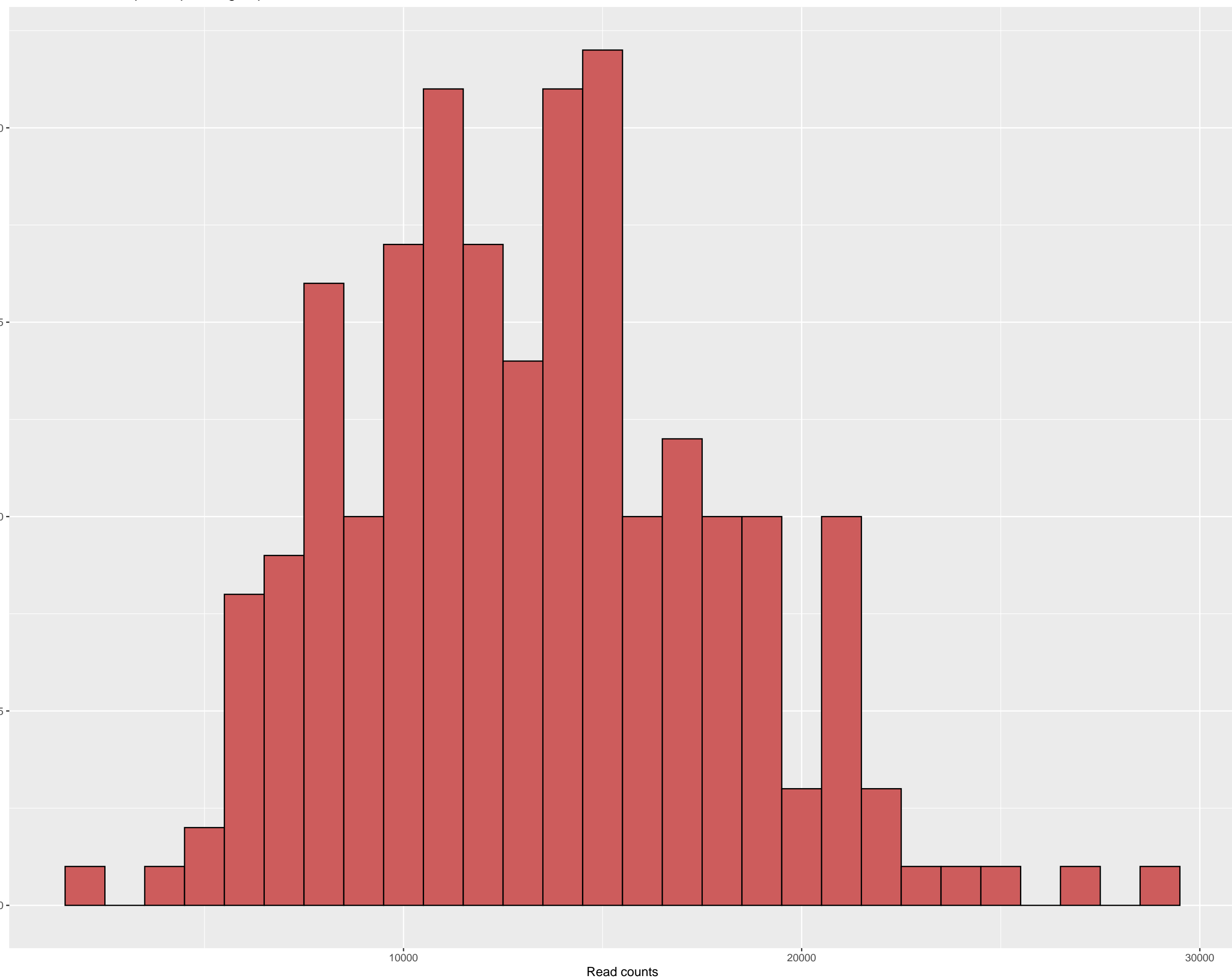
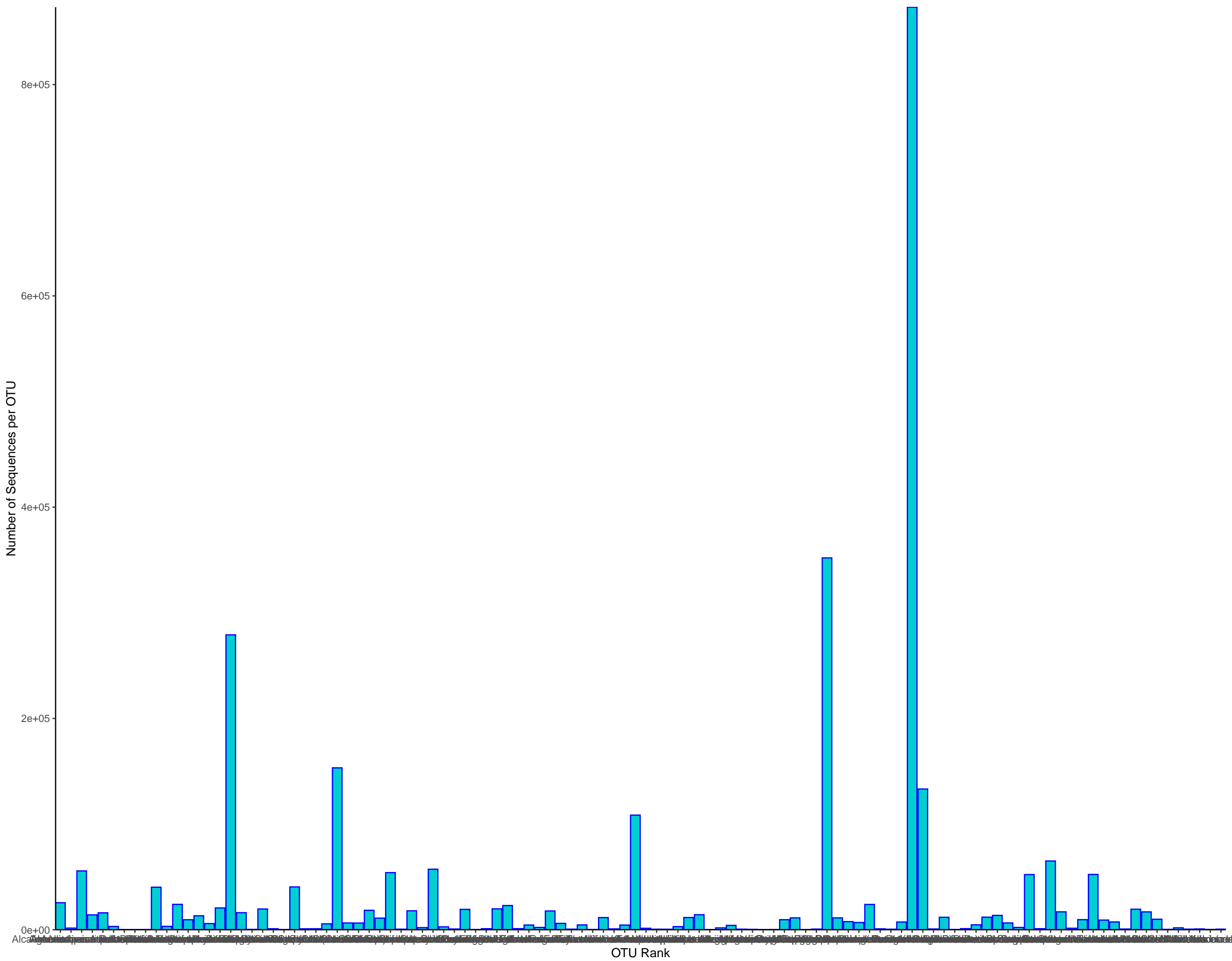


1.Explore your Data (Sequencing depths)

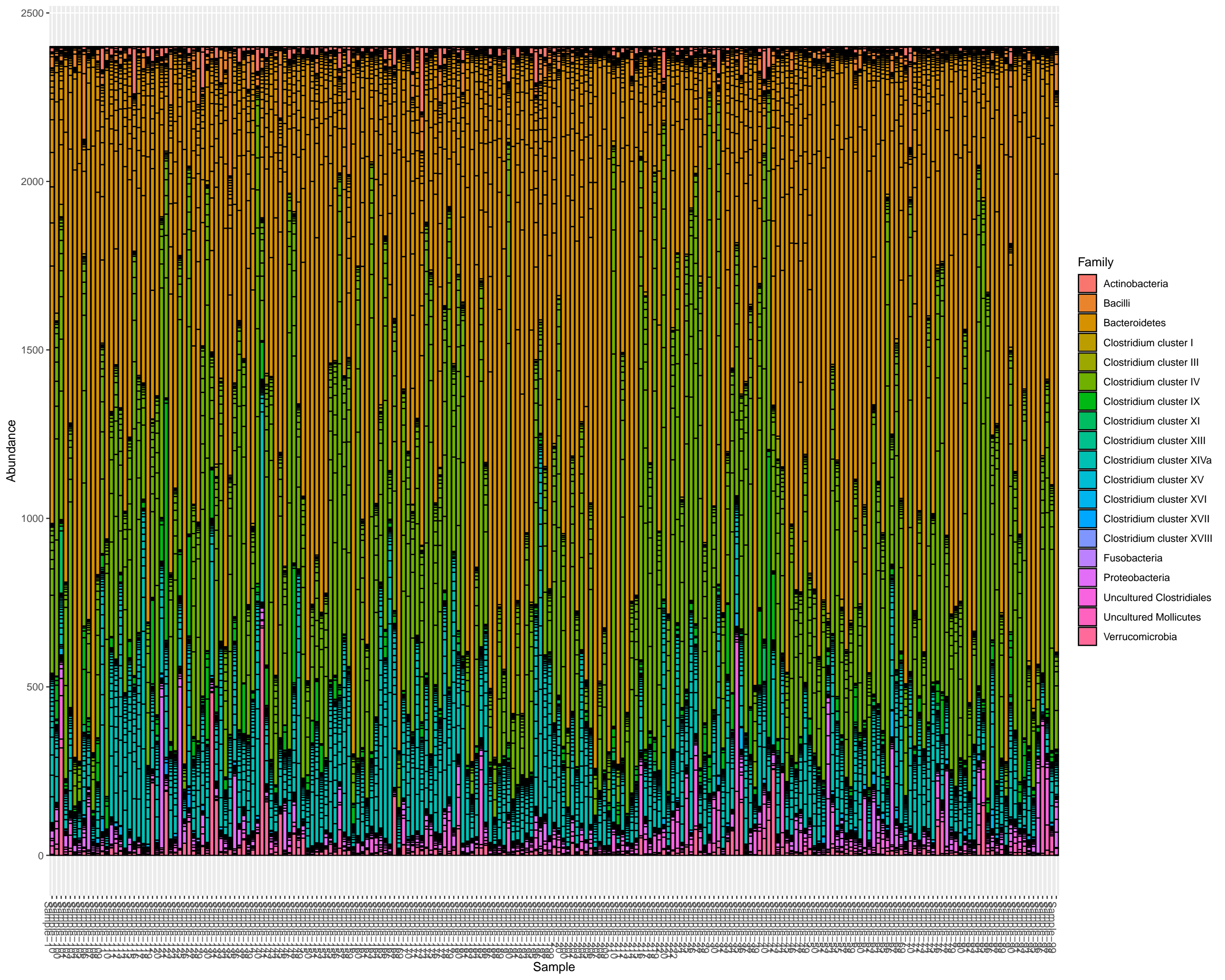
Distribution of sample sequencing depth



Rank Abundance Curve of the OTUs



2.Alpha diversity



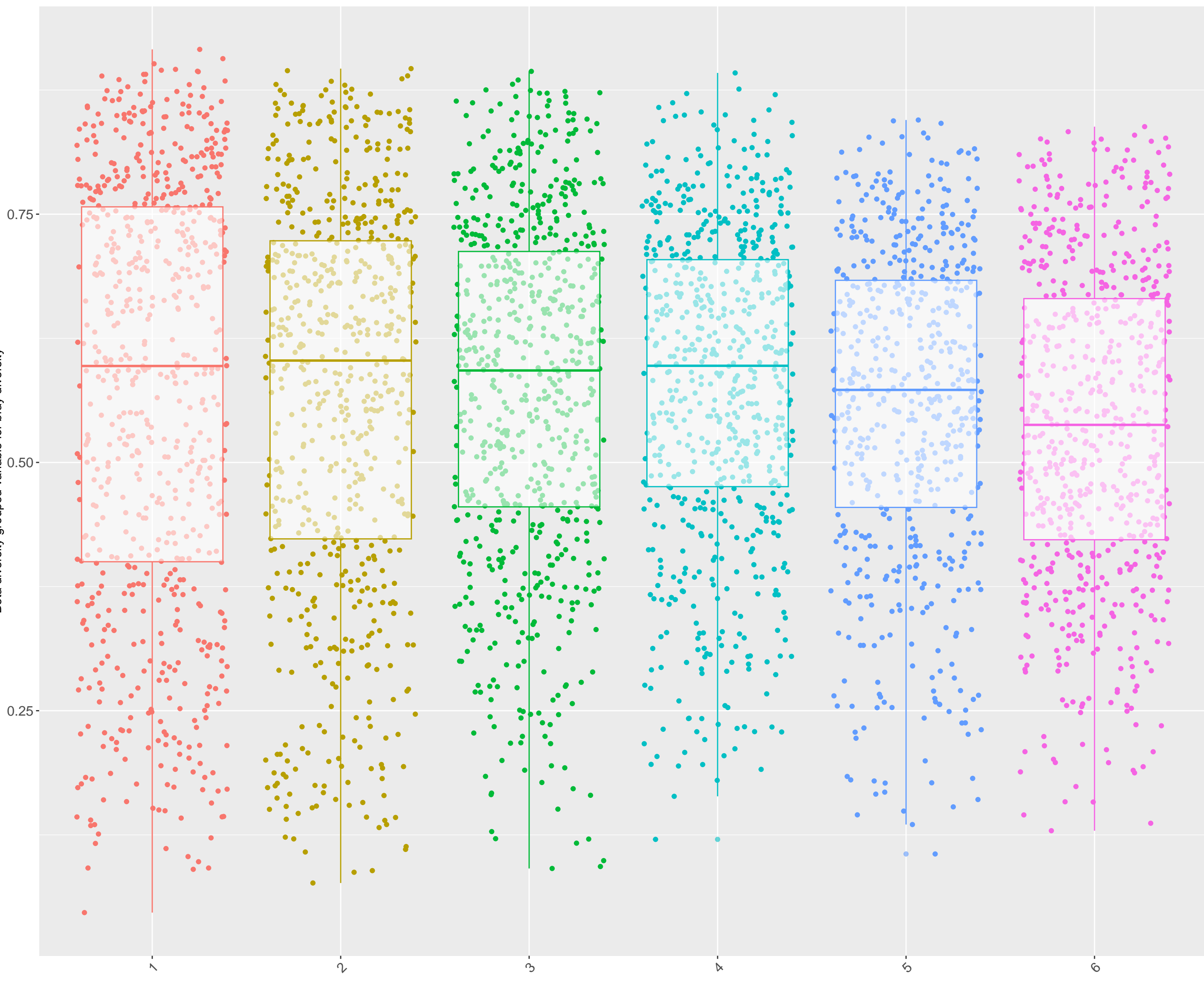
| | | | | | | | | | |
|------------|----|------------------|------------------|------------------|------------------|------------------|-------------------|------------------|------------------|
| Sample.90 | 81 | 94.1538461538462 | 7.99281608090598 | 96.5374107165347 | 4.79679421243531 | 2.41015578791334 | 0.773992013888889 | 4.42462240917617 | 16.1800572404844 |
| Sample.91 | 77 | 111.363636363636 | 17.5151861865188 | 108.637667485843 | 5.37136940616098 | 1.92350914916903 | 0.691065972222222 | 3.23693704831803 | 15.1908553378196 |
| Sample.92 | 81 | 123.166666666667 | 24.7366715159883 | 100.090877558099 | 4.82376494030302 | 2.38399119406156 | 0.808827777777778 | 5.23088547266862 | 16.1800572404844 |
| Sample.93 | 80 | 91.4 | 6.95458630358584 | 94.3264349735138 | 4.50572424146085 | 1.77848857500852 | 0.569696875 | 2.32394315054068 | 15.9313309709258 |
| Sample.94 | 72 | 142.2 | 40.1888758460865 | 97.9055489964581 | 4.78133217223914 | 1.4053853225723 | 0.485121875 | 1.94220719709154 | 13.9760027012311 |
| Sample.95 | 71 | 90.25 | 10.8677492761215 | 92.2431808341752 | 5.01601009164139 | 1.63482299640724 | 0.562411805555556 | 2.28525360760609 | 13.7359652427869 |
| Sample.96 | 70 | 97.1428571428571 | 16.5005915061527 | 89.1197741423986 | 4.67204358748537 | 2.39902876662598 | 0.807578819444444 | 5.19693308768201 | 13.4969171154013 |
| Sample.97 | 73 | 101.5 | 17.9317946977915 | 89.1224473314607 | 4.68401264466688 | 2.49178232743777 | 0.825075 | 5.71673574389024 | 14.217024368527 |
| Sample.98 | 72 | 93.8571428571429 | 13.8797777941804 | 85.6596358391923 | 4.46770405310295 | 2.24410182963486 | 0.764839236111111 | 4.25241006817145 | 13.9760027012311 |
| Sample.99 | 77 | 87.4615384615385 | 6.75236737312226 | 88.6143911105548 | 4.37235075537642 | 1.9889502735934 | 0.637246875 | 2.75669575555 | 15.1908553378196 |
| Sample.100 | 90 | 107.1 | 10.4104915657486 | 104.205642479214 | 5.03032314397663 | 3.24018405628126 | 0.930360069444444 | 14.3595777885253 | 18.460603486945 |
| Sample.101 | 74 | 101.083333333333 | 14.1566586815387 | 101.995196284364 | 5.11310897255449 | 1.84617304171175 | 0.660489236111111 | 2.94541471541464 | 14.4590252584172 |
| Sample.102 | 82 | 101 | 11.2896737186363 | 98.4343230352769 | 4.8302297928907 | 2.46694478255421 | 0.810669444444444 | 5.28176763156736 | 16.4297271894329 |
| Sample.103 | 68 | 75.5555555555556 | 4.98247153772291 | 80.6757731132831 | 4.03957891921893 | 1.32948227245661 | 0.458407291666667 | 1.84640595158185 | 13.0218100540161 |
| Sample.104 | 72 | 103.909090909091 | 16.5104041068042 | 100.340954888082 | 5.01637714027634 | 1.28685078203355 | 0.439954513888889 | 1.78556925249747 | 13.9760027012311 |
| Sample.105 | 78 | 89.1428571428571 | 8.22978385953756 | 85.8124017957351 | 4.49287150790469 | 3.07355439924135 | 0.919676388888889 | 12.4496394791901 | 15.4367258058891 |
| Sample.106 | 73 | 90.1 | 10.4098928658444 | 87.4619230769231 | 4.42194085877114 | 1.97246386190172 | 0.646677777777778 | 2.8302776816881 | 14.217024368527 |
| Sample.107 | 71 | 82.0526315789474 | 6.44784762386685 | 88.3459611389466 | 4.49084775738624 | 1.2389687539107 | 0.425423611111111 | 1.740412622826 | 13.7359652427869 |
| Sample.108 | 72 | 81.5454545454545 | 6.56584199387946 | 83.3172095761382 | 4.46717610687544 | 2.38604572040022 | 0.757011458333333 | 4.11542039448019 | 13.9760027012311 |
| Sample.109 | 89 | 123.5 | 19.2744333180455 | 113.246971292679 | 5.15318216537166 | 3.39838447843337 | 0.946685069444444 | 18.7564719597257 | 18.2035189631603 |
| Sample.110 | 81 | 107.25 | 15.469776401633 | 104.349375 | 5.22169607537528 | 2.84674120191628 | 0.860159375 | 7.15099778766006 | 16.1800572404844 |
| Sample.111 | 79 | 106.142857142857 | 16.5013361628345 | 101.588300702911 | 5.07364746869764 | 3.145034859698 | 0.919183333333333 | 12.3736852959373 | 15.6835524391416 |
| Sample.112 | 77 | 83 | 4.69857113873921 | 88.7043261749144 | 4.77671996612468 | 3.0364962037445 | 0.875061111111111 | 8.00391302414514 | 15.1908553378196 |
| Sample.113 | 68 | 88 | 13.4939193189325 | 85.4331551628849 | 4.74274237728147 | 2.53813751785101 | 0.795871527777778 | 4.89887564000068 | 13.0218100540161 |
| Sample.114 | 73 | 84.1428571428571 | 8.22952658528904 | 83.9274394160584 | 4.61331439364544 | 2.91513815151746 | 0.870756944444444 | 7.73735962602762 | 14.217024368527 |
| Sample.115 | 76 | 99.3333333333333 | 13.6351405964948 | 96.5829643019515 | 4.83981285685687 | 2.81614348552458 | 0.874343055555556 | 7.95817536723663 | 14.9459454111693 |
| Sample.116 | 78 | 108.6 | 20.0084984154179 | 94.2349796407186 | 4.86984799268934 | 3.13192082983615 | 0.910368402777778 | 11.1567798744087 | 15.4367258058891 |
| Sample.117 | 76 | 88 | 7.95955686295069 | 91.5913128924856 | 4.88629103442906 | 3.04987823376226 | 0.908839583333333 | 10.9696734236808 | 14.9459454111693 |
| Sample.118 | 67 | 91.4285714285714 | 15.1641965083449 | 83.8371313518119 | 4.49133136855826 | 1.98031855274714 | 0.653907291666667 | 2.8893992156485 | 12.7857620926352 |
| Sample.119 | 76 | 82 | 4.69855253114465 | 84.3278612890689 | 4.5437088267295 | 3.06497037295816 | 0.9167375 | 12.0102086773758 | 14.9459454111693 |
| Sample.120 | 82 | 92.4615384615385 | 6.75246477403439 | 94.9379736665425 | 4.74550925356807 | 2.72235049447772 | 0.861513194444444 | 7.22090451858129 | 16.4297271894329 |
| Sample.121 | 81 | 90.5454545454545 | 6.56606487850268 | 91.7443621876732 | 4.70484967631208 | 2.84598455150714 | 0.873002083333333 | 7.87414491707541 | 16.1800572404844 |
| Sample.122 | 82 | 86.7894736842105 | 3.60509087799287 | 92.1389126566978 | 4.63102434732679 | 2.7364983402415 | 0.84858125 | 6.60420192347381 | 16.4297271894329 |
| Sample.123 | 75 | 85.5 | 7.20569949853294 | 85.6106629240974 | 4.51835086498336 | 2.53640776289402 | 0.820305208333333 | 5.56499156555966 | 14.702000515631 |
| Sample.124 | 82 | 90 | 5.24857041007177 | 96.8936541193182 | 4.9255910983418 | 2.63935917102687 | 0.851982291666667 | 6.75594840144408 | 16.4297271894329 |
| Sample.125 | 64 | 83.125 | 12.0463579801871 | 78.5064456696152 | 4.17652754781938 | 1.54135428732682 | 0.550248611111111 | 2.22345061005926 | 12.08370965492 |
| Sample.126 | 85 | 155.2 | 40.1909294467522 | 118.658151134255 | 5.67485263085648 | 3.02901115878708 | 0.89914375 | 9.91510193964182 | 17.1843599948278 |
| Sample.127 | 78 | 87.4285714285714 | 7.2557718981011 | 86.0337719298246 | 4.57808387538408 | 3.13654374415627 | 0.916215972222222 | 11.9354491127154 | 15.4367258058891 |
| Sample.128 | 79 | 86 | 5.0560298117196 | 88.0175709914496 | 4.46537207129891 | 2.90478399824335 | 0.897810416666667 | 9.78573321644819 | 15.6835524391416 |
| Sample.129 | 75 | 110 | 25.6088733438118 | 86.2817944187269 | 4.56850117787285 | 3.11270902144616 | 0.907735069444444 | 10.8383542260173 | 14.702000515631 |
| Sample.130 | 78 | 101.333333333333 | 13.6352435629965 | 98.3128071411944 | 4.99525329304259 | 2.93255240382074 | 0.906525694444444 | 10.6981270848347 | 15.4367258058891 |
| Sample.131 | 75 | 86.375 | 8.08289606816159 | 85.8272390886312 | 4.60603281046071 | 2.80322964291892 | 0.852432986111111 | 6.77658220253039 | 14.702000515631 |
| Sample.132 | 79 | 96.1 | 10.4101283627348 | 100.056899581024 | 5.14811576423854 | 2.93649811199503 | 0.88216875 | 8.48671298997507 | 15.6835524391416 |
| Sample.133 | 76 | 85.5454545454545 | 6.565946844549 | 85.9770425902478 | 4.40590419321262 | 2.27157472503843 | 0.716025347222222 | 3.5214410519327 | 14.9459454111693 |

Test whether the observed number of OTUs differs significantly between the variable using Wilcoxon rank–sum test

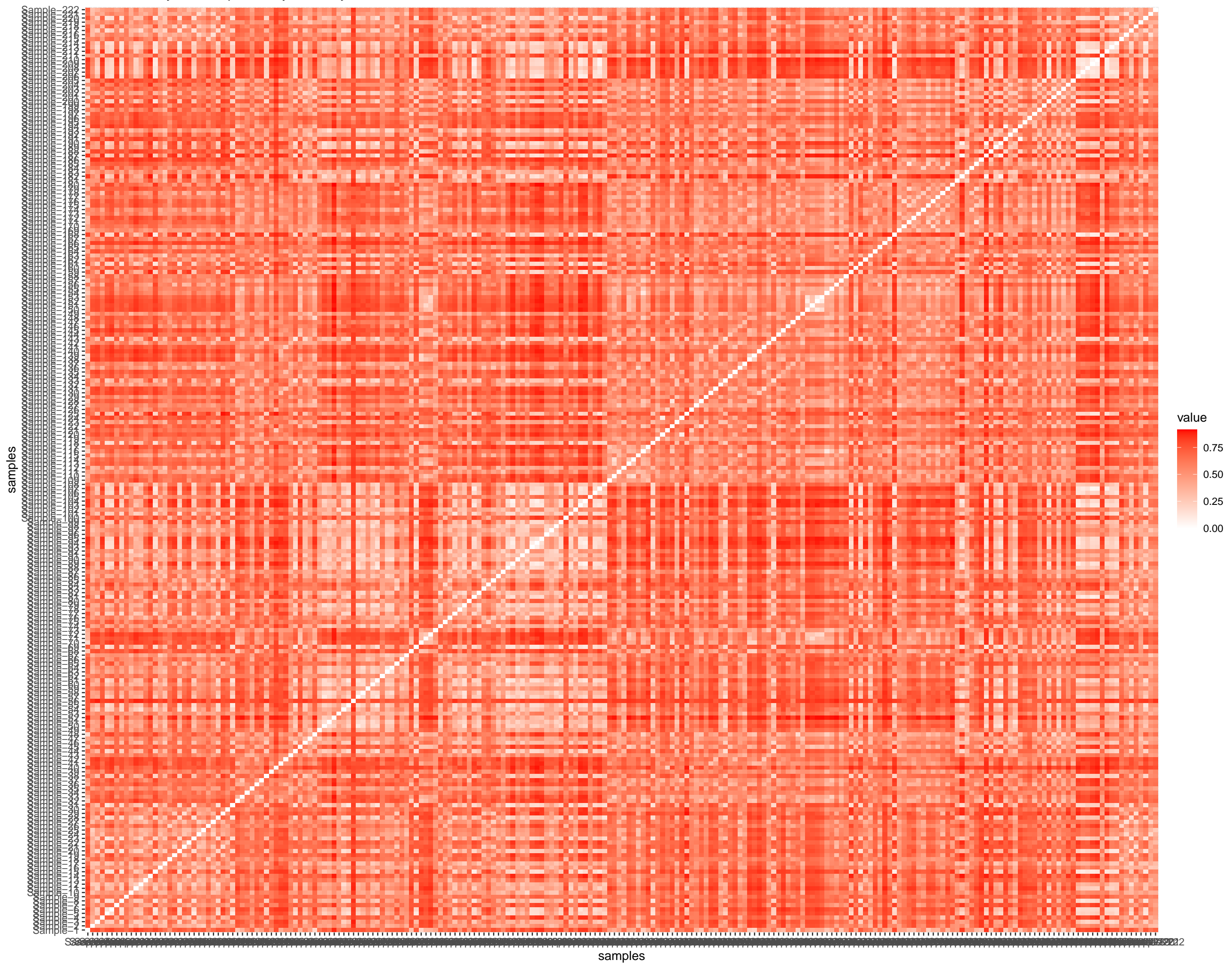
| | 1 | 2 | 3 | 4 | 5 |
|---|-------------------|-------------------|-------------------|----|----|
| 2 | 1 | NA | NA | NA | NA |
| 3 | 1 | 1 | NA | NA | NA |
| 4 | 0.654695752094996 | 0.167010245153008 | 0.349530052028851 | NA | NA |
| 5 | 1 | 1 | 1 | 1 | NA |
| 6 | 1 | 0.22698515648416 | 0.523084175396183 | 1 | 1 |

3. Beta diversity

Beta diversity grouped variable for bray diversity

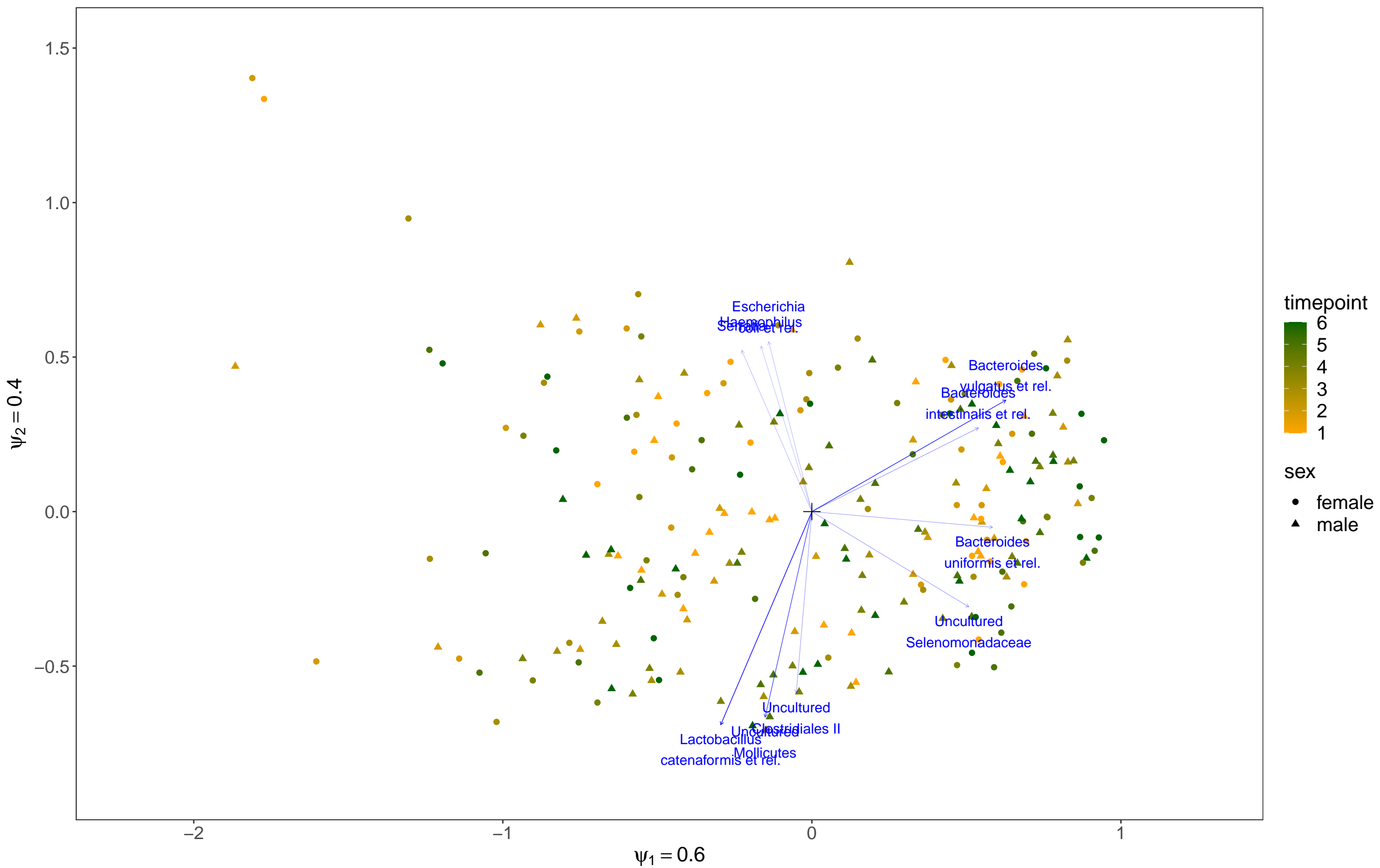


Beta diversity heatmap for bray diversity

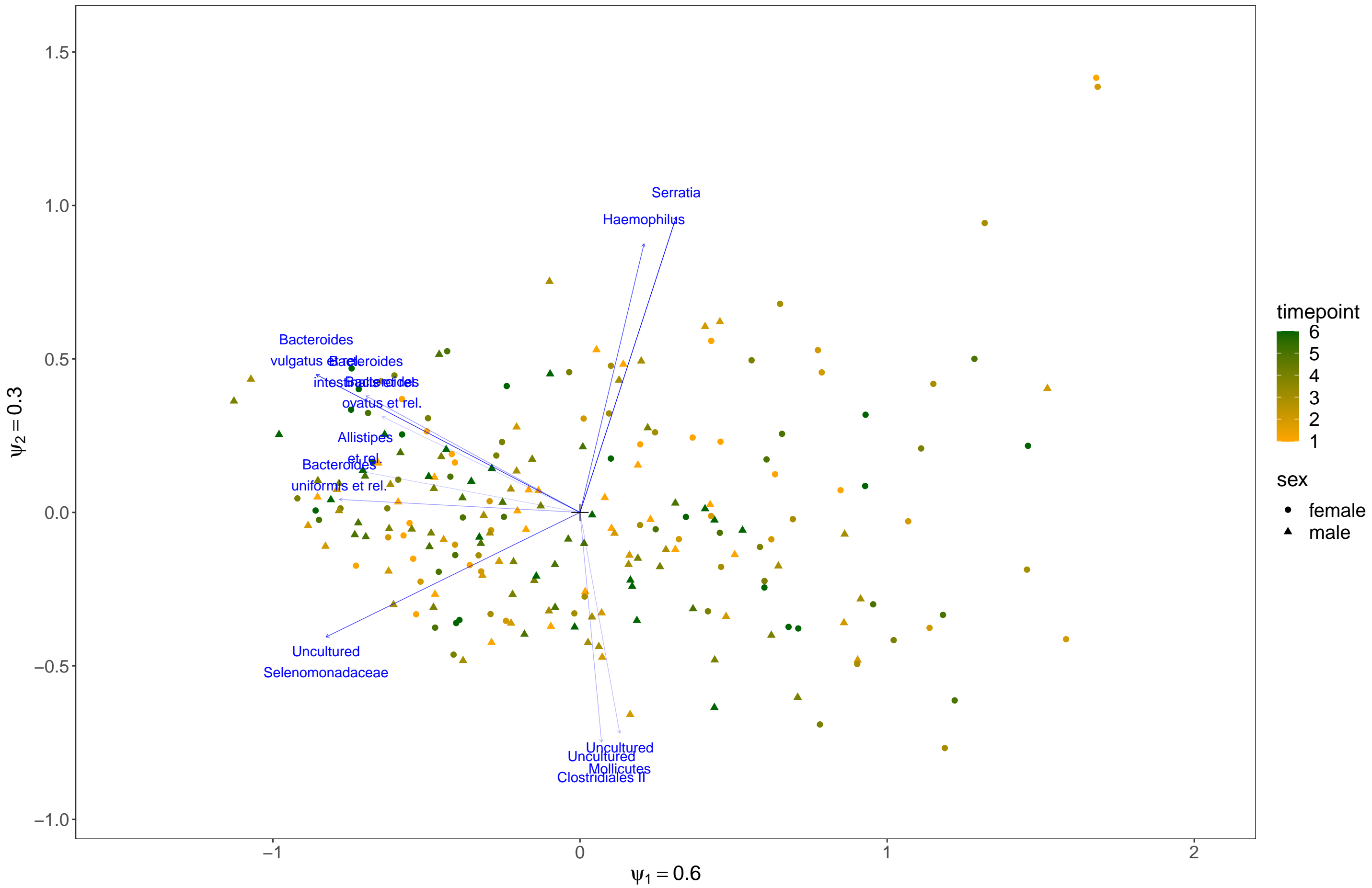


4. Ordination techniques

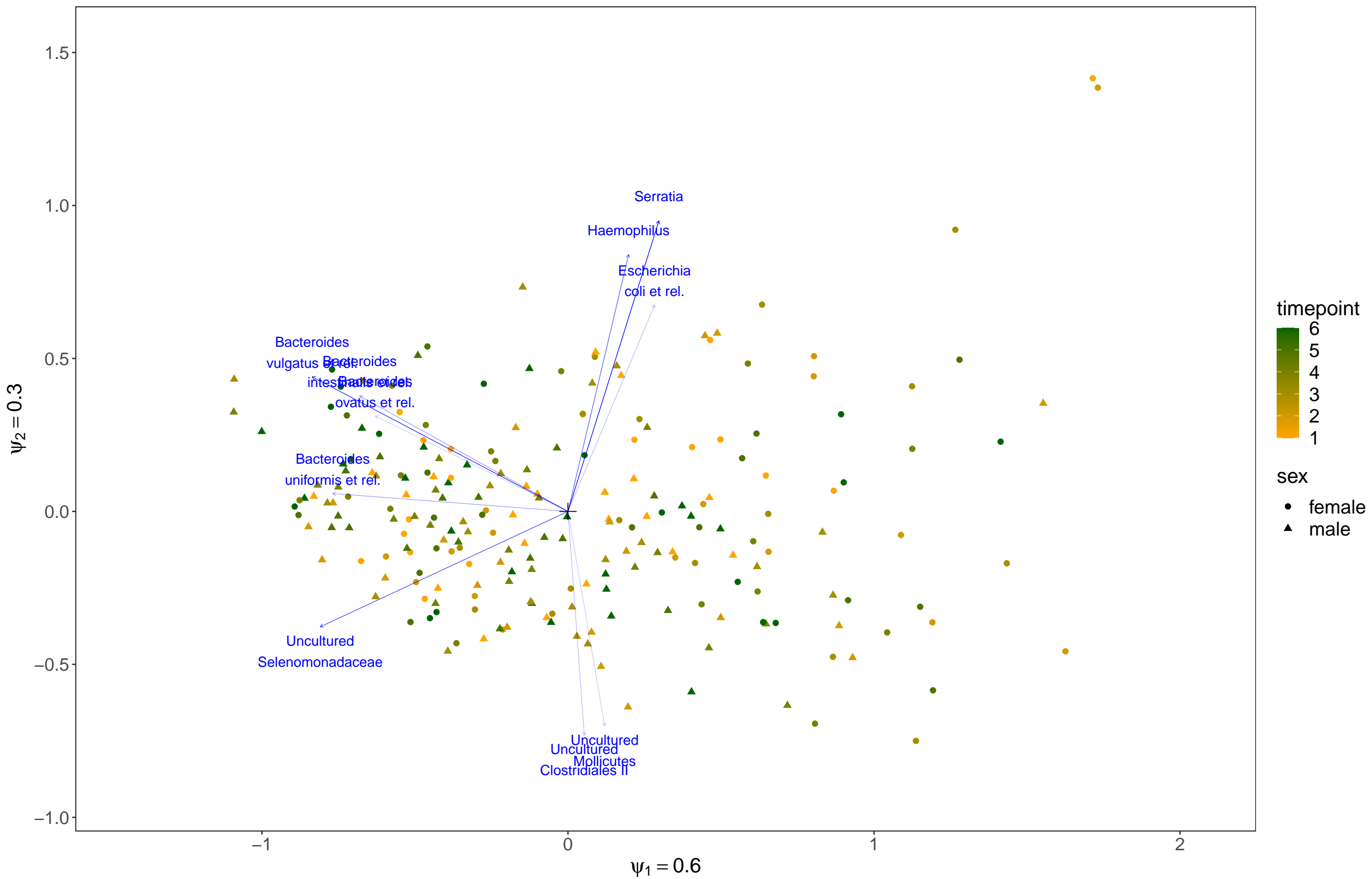
Fit Unconstrained RCM



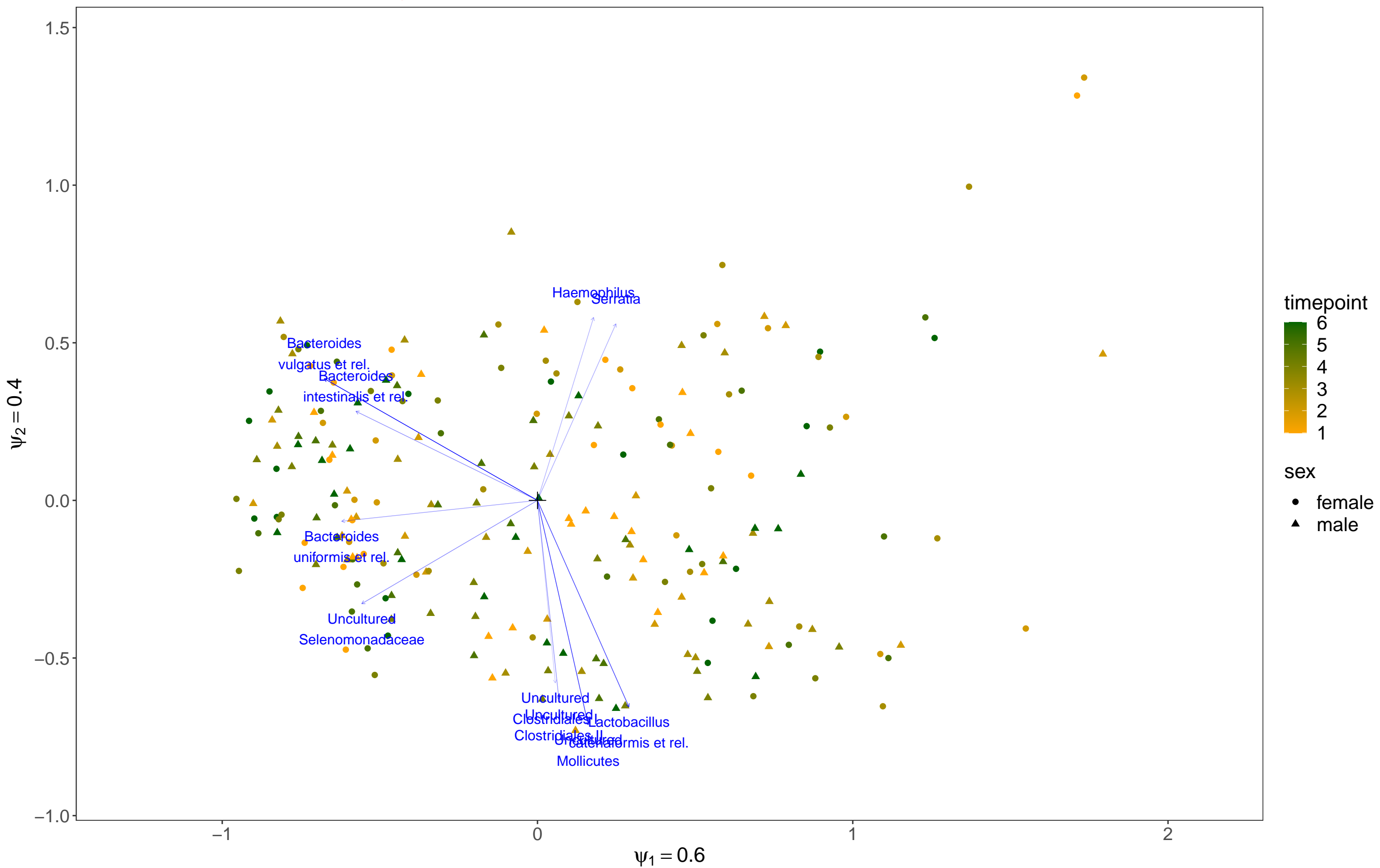
Total confounders RCM



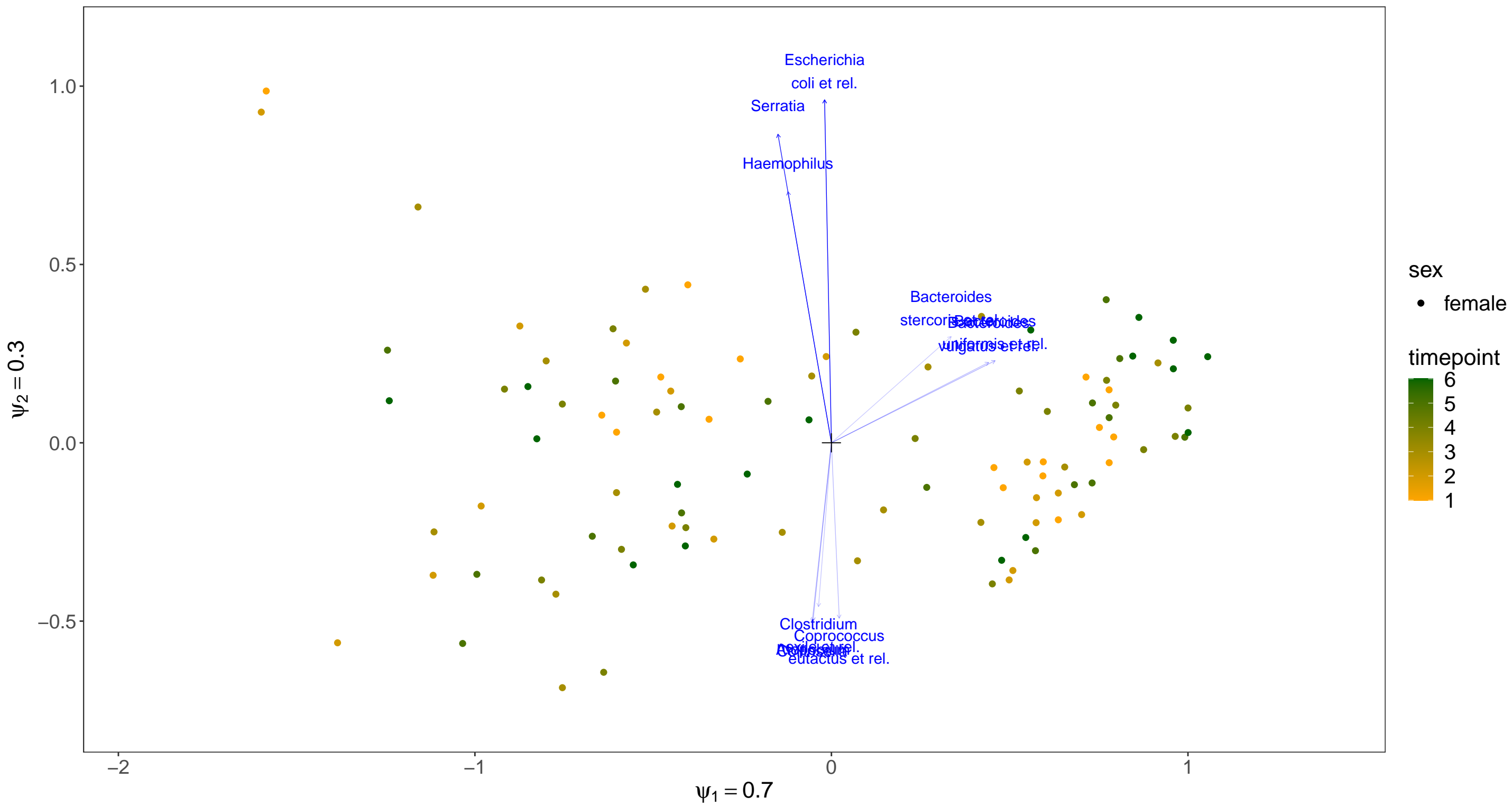
RCM for confounder bmi_group



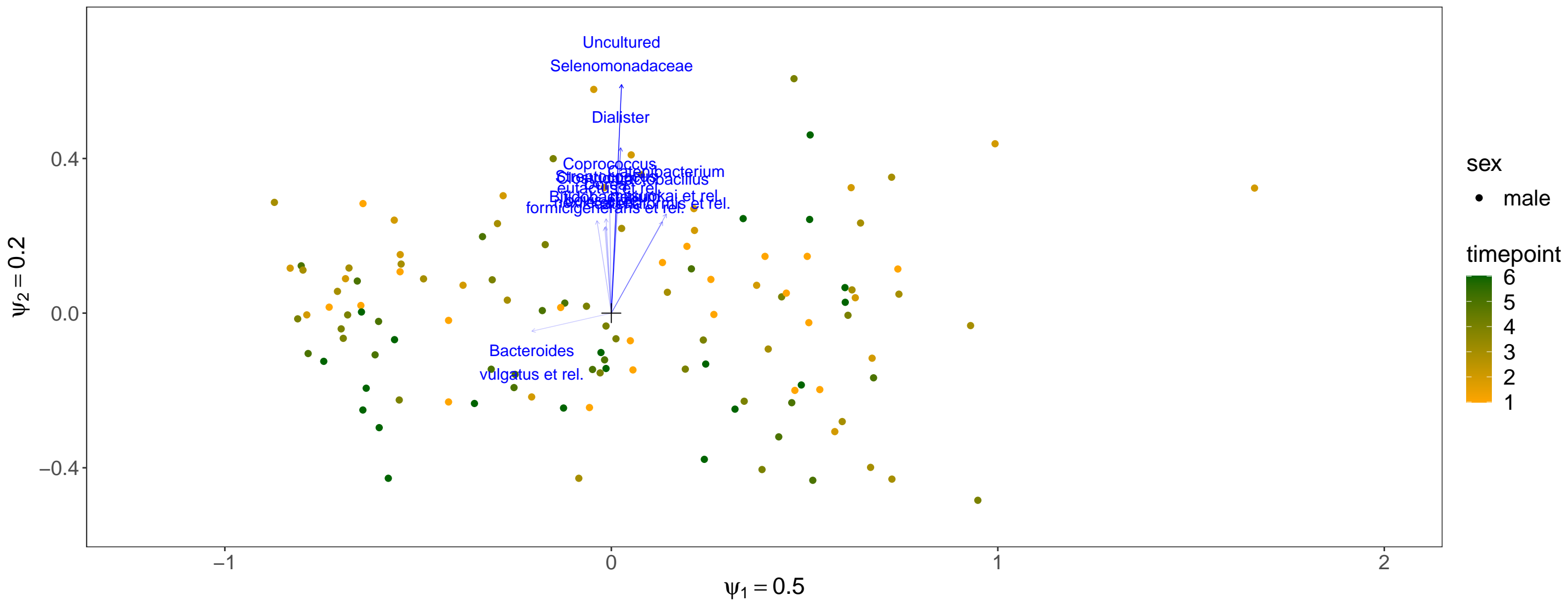
RCM for confounder timepoint.within.group

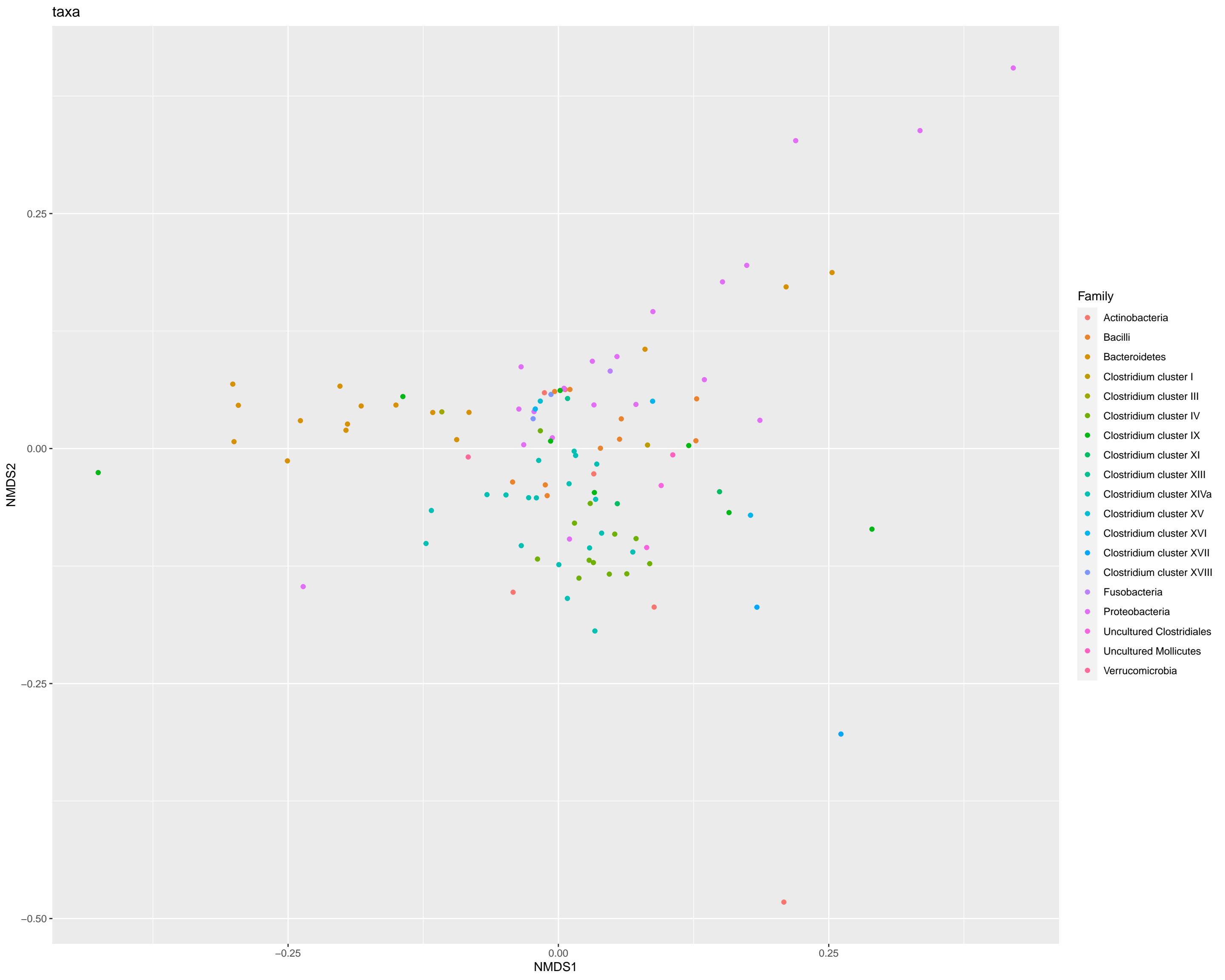


RCM split sex to female

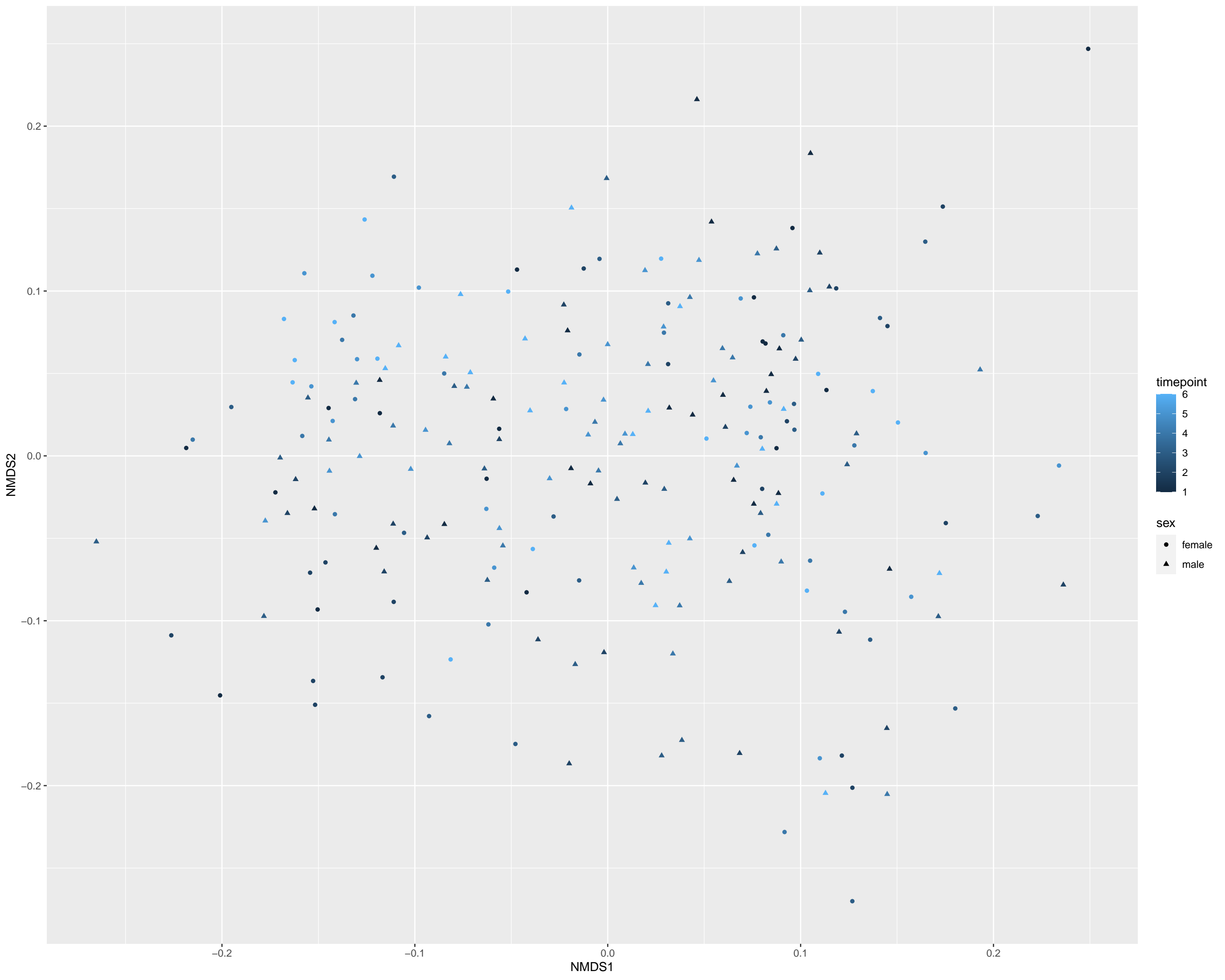


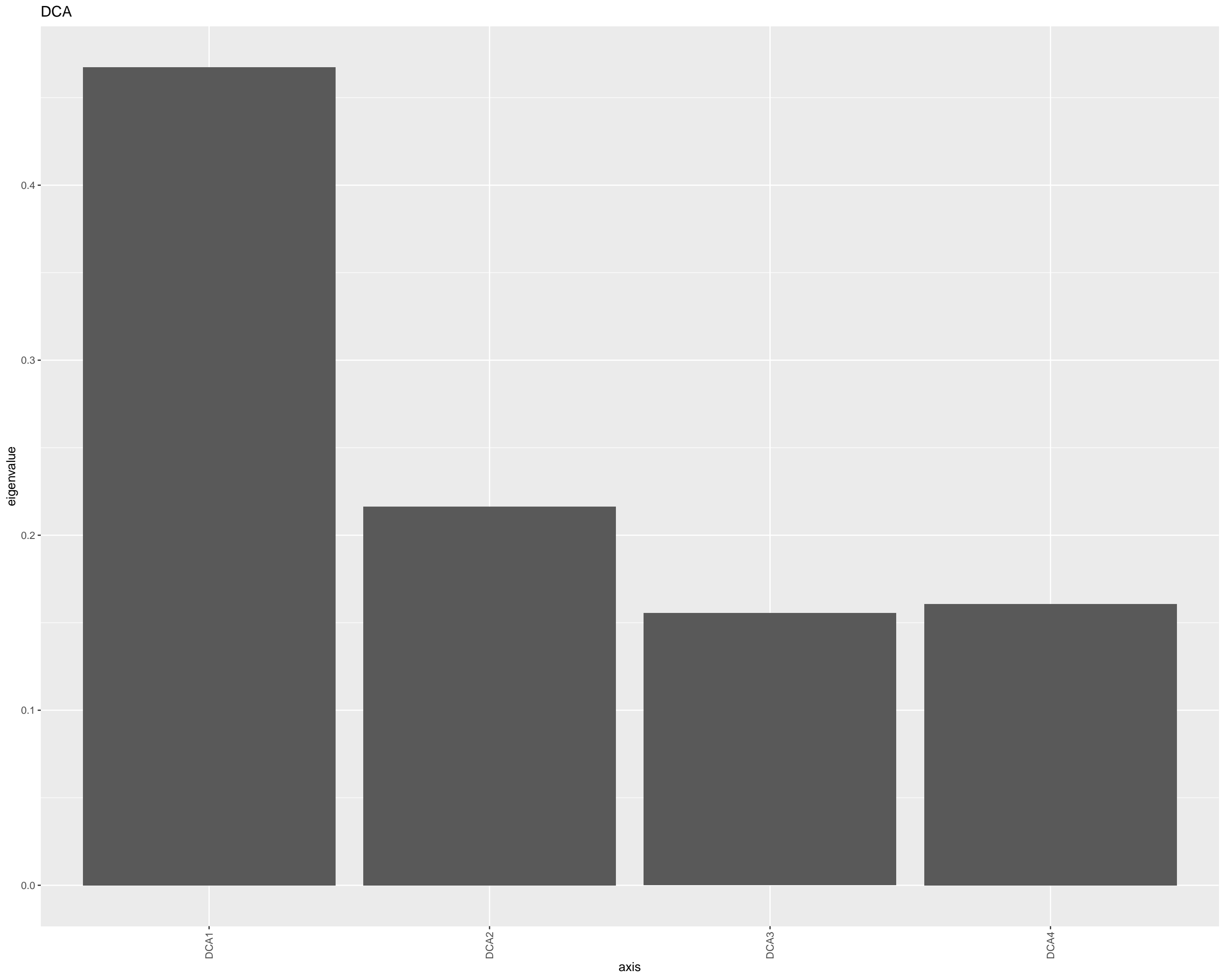
RCM split sex to male



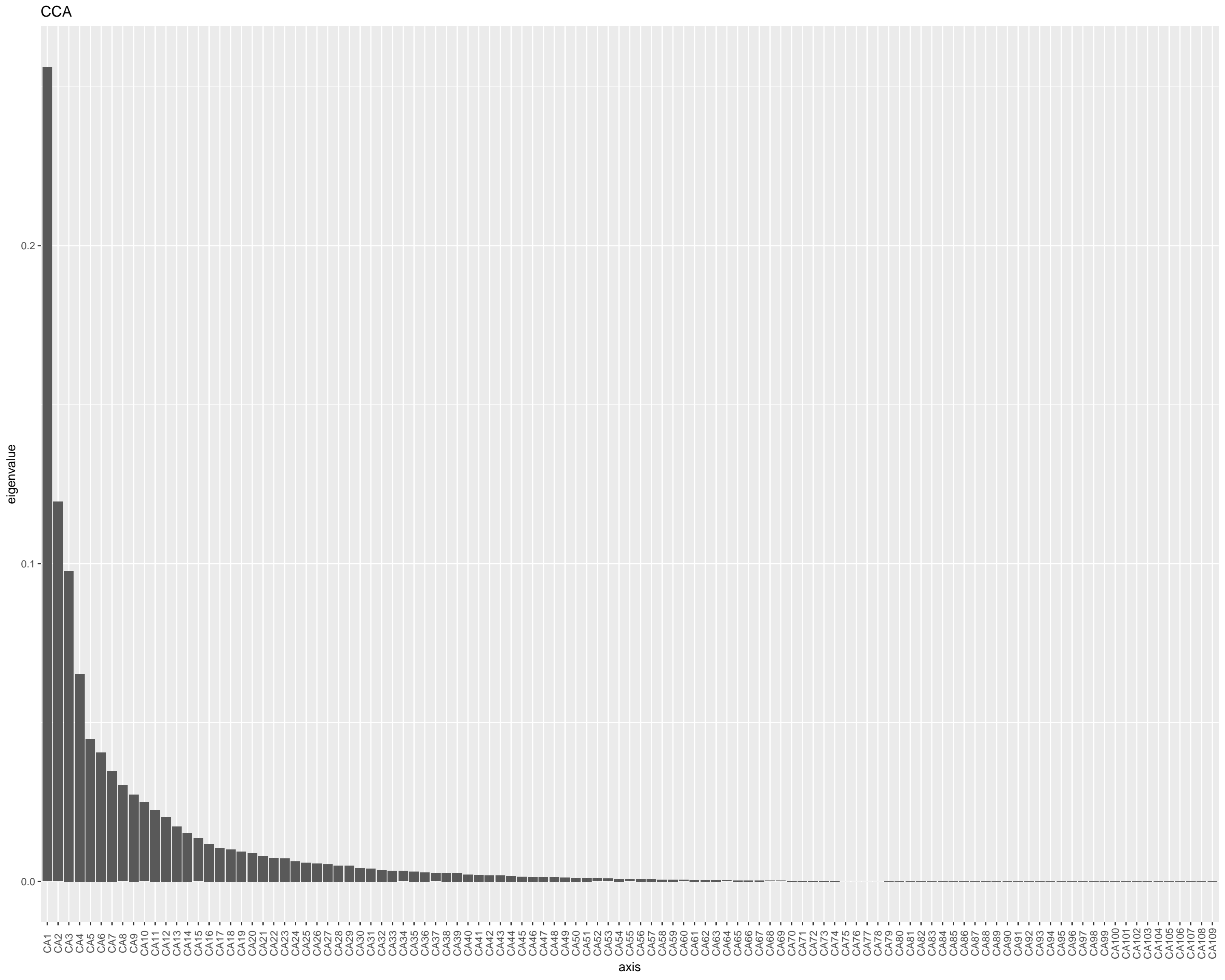


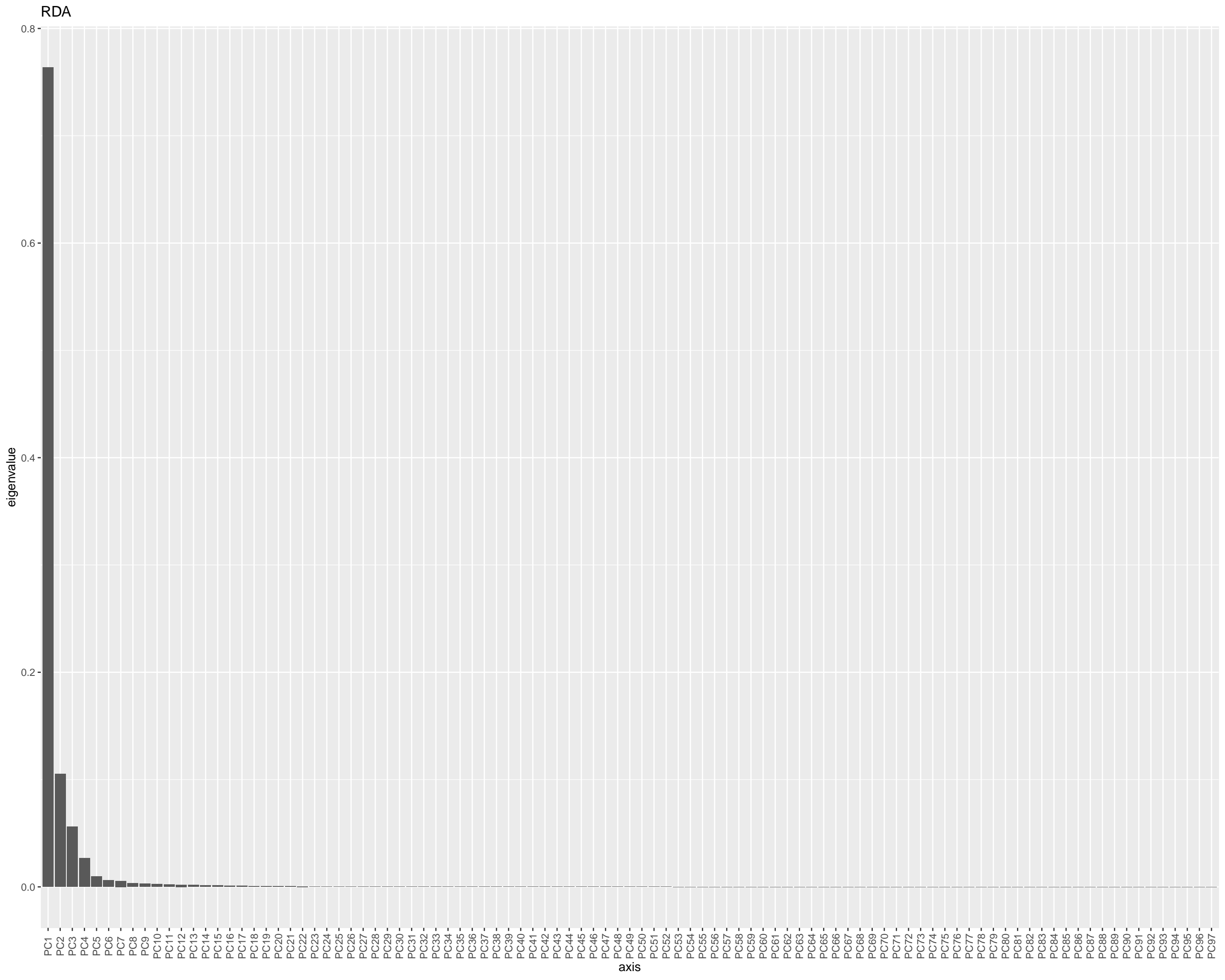




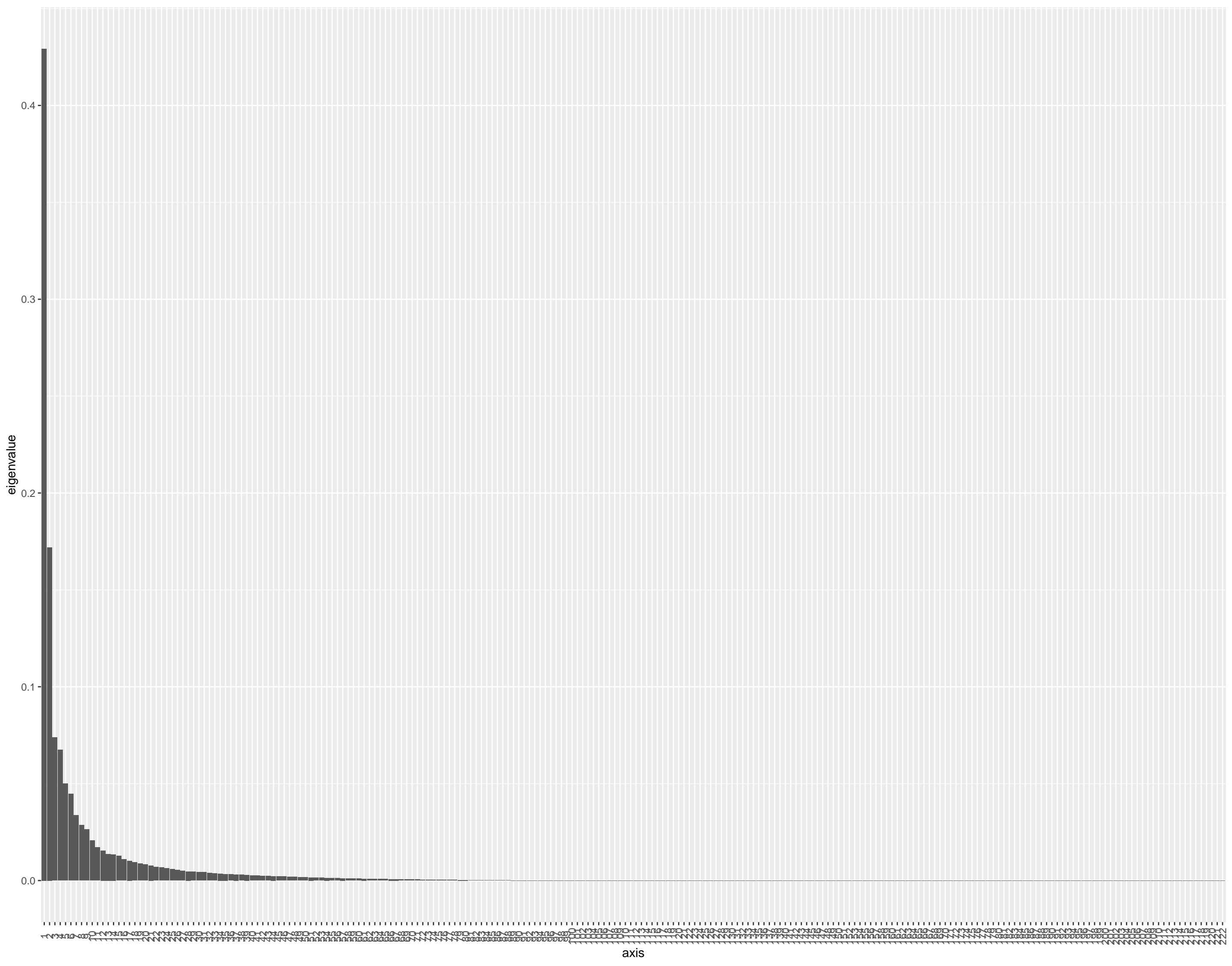


CCA

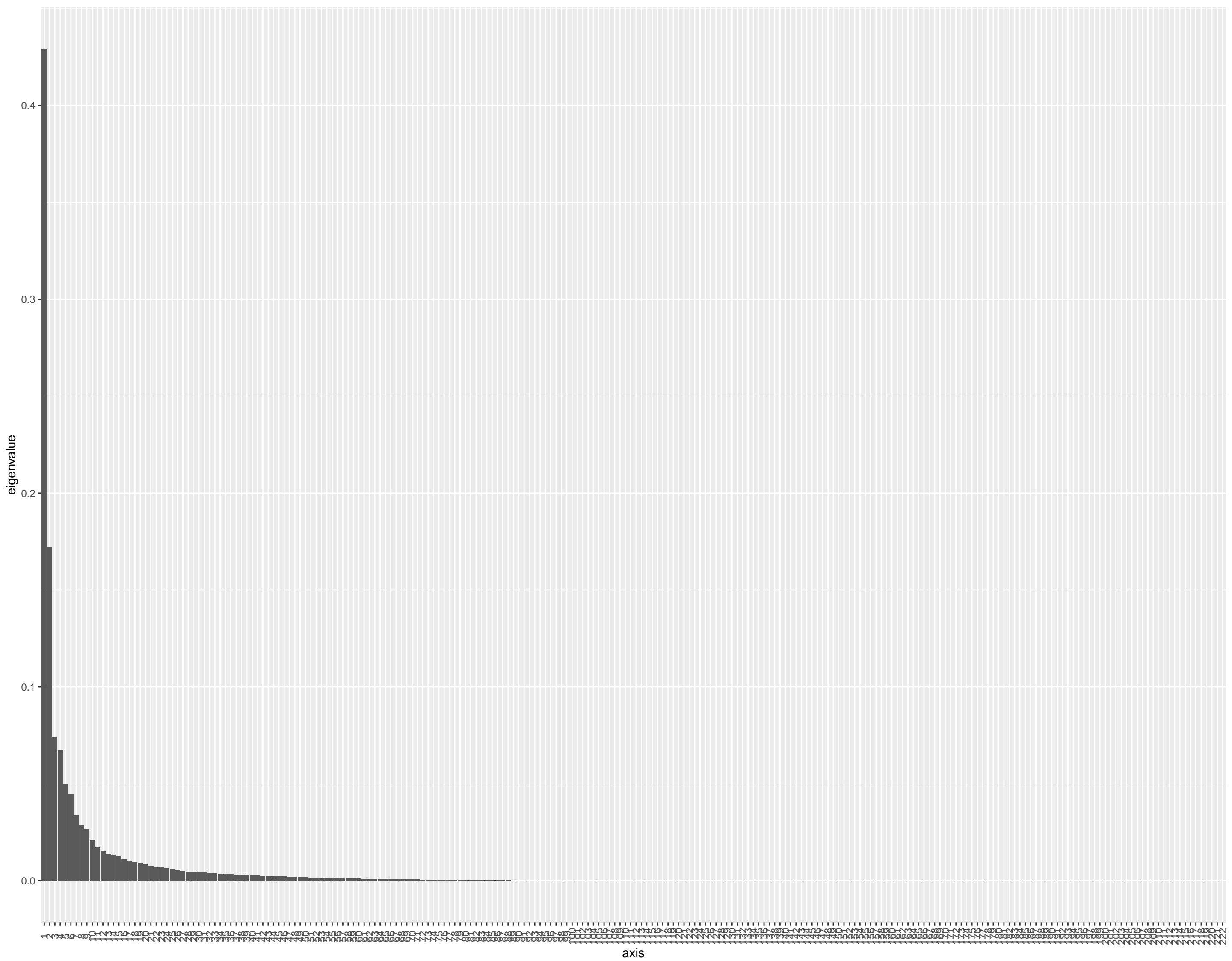




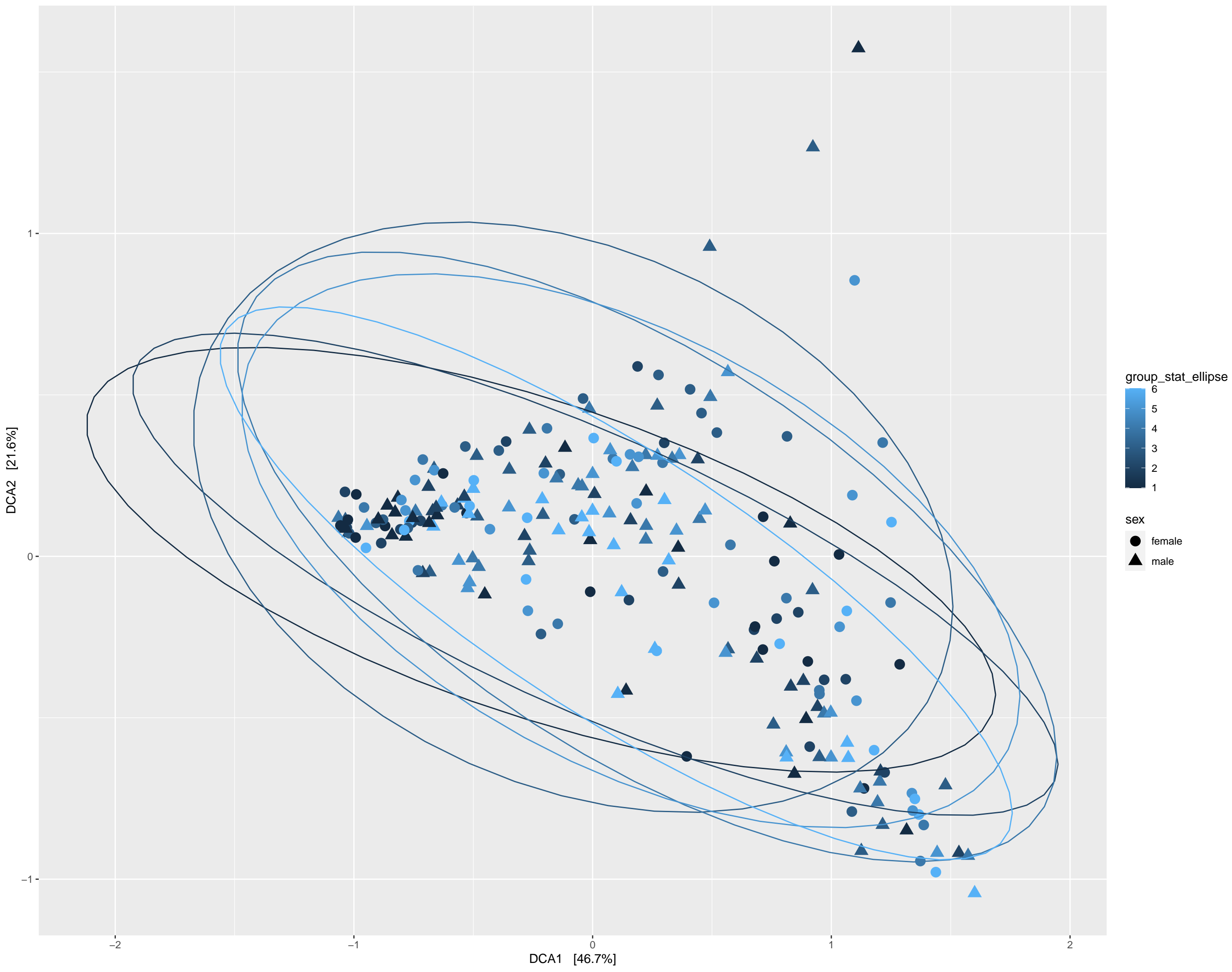
MDS



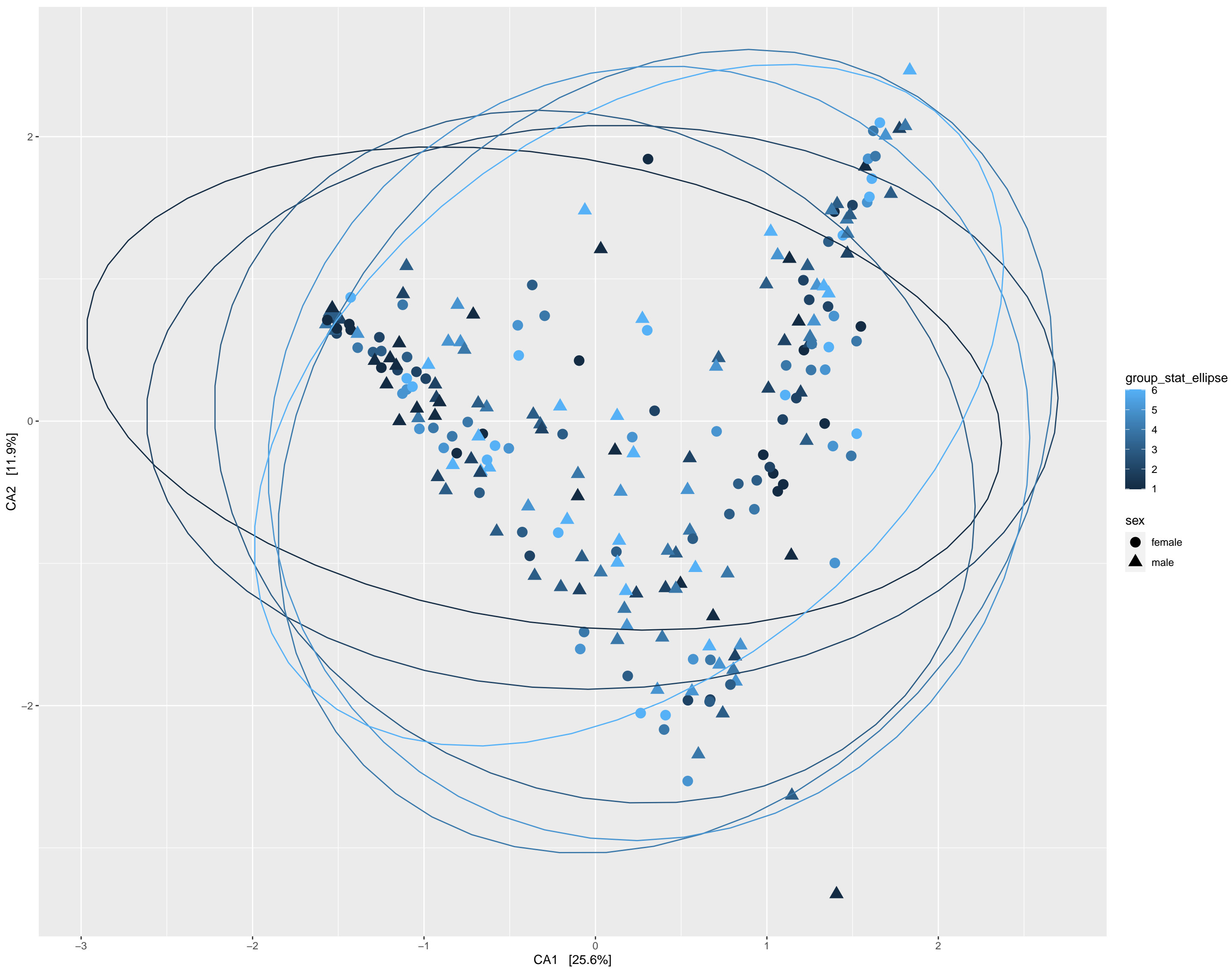
PCoA



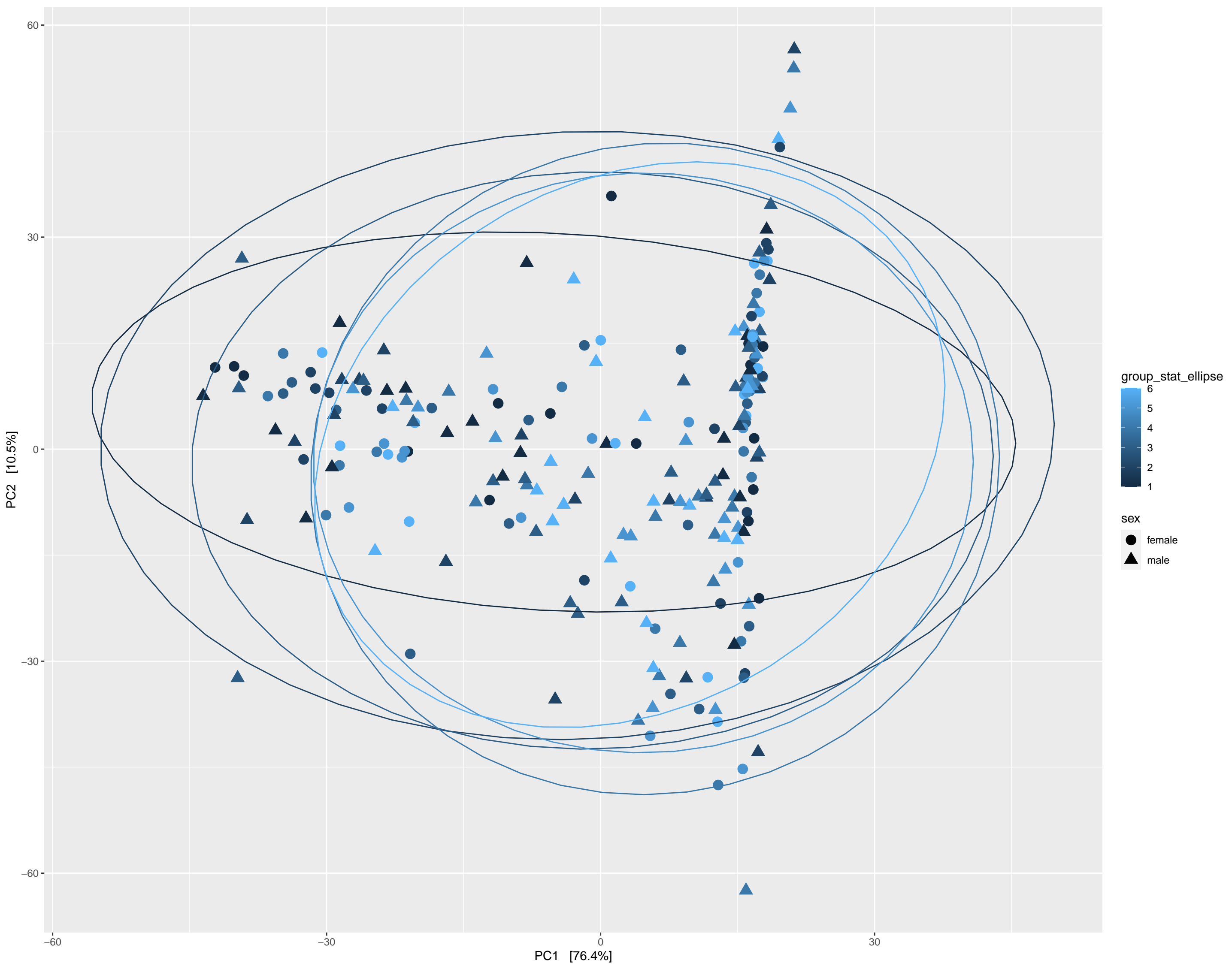
DCA



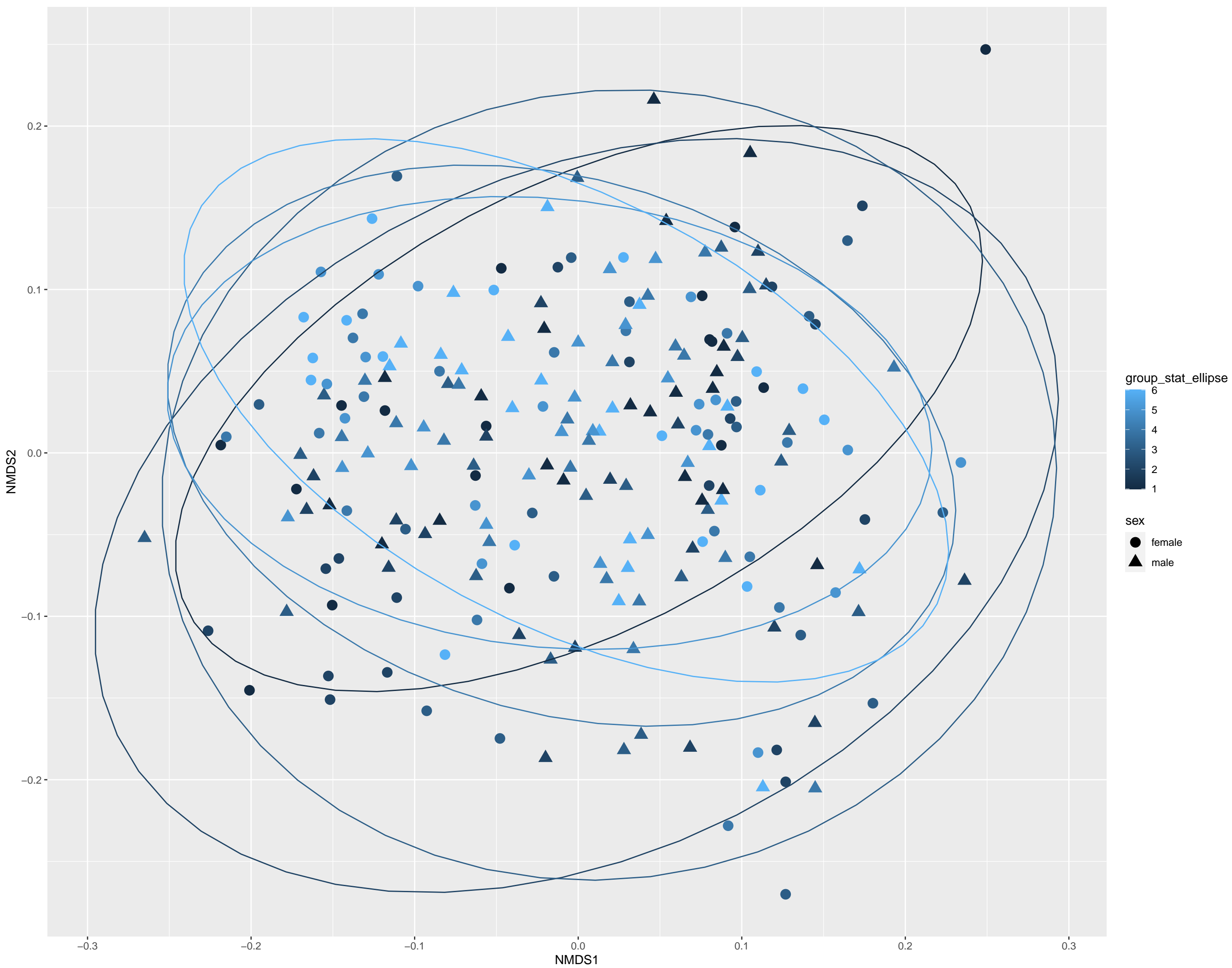
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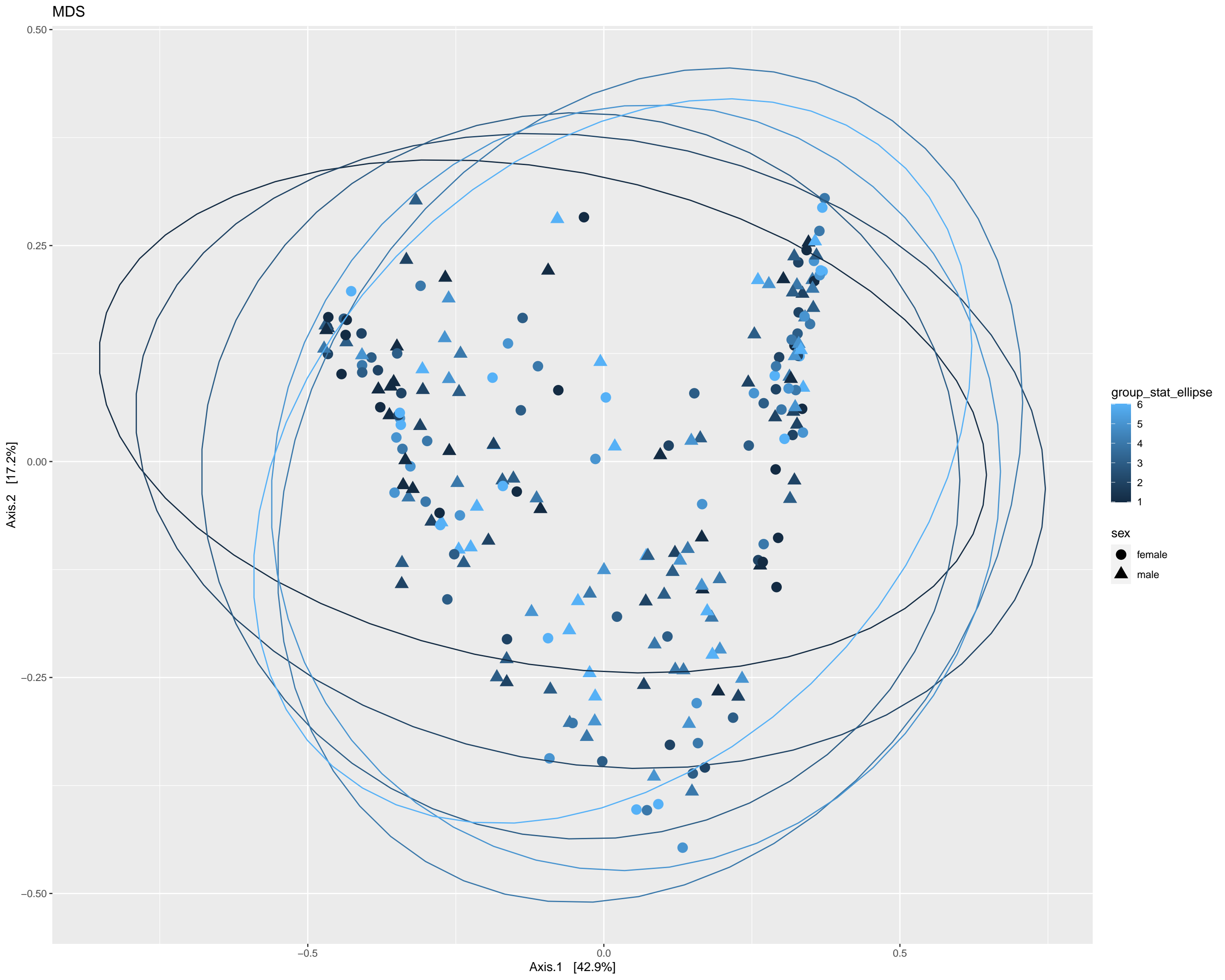


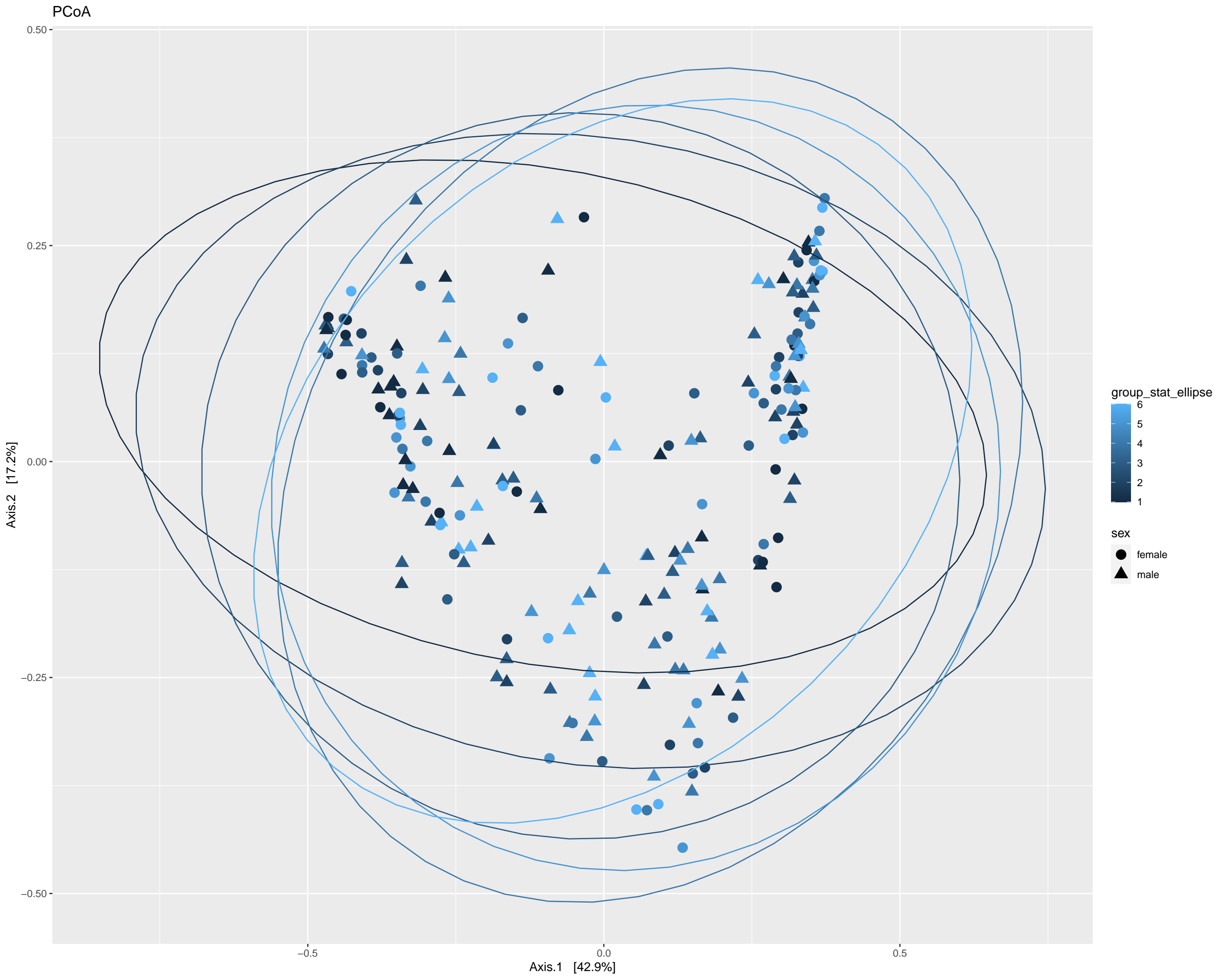
RDA



NMDS







5.Null hypothesis