MantelProcrustes

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Walking through Mantel/Procrustes

Start by reading in microbiome and metabolite data:

```
metabolite = read.csv("./data/metabolite.csv")
tomelt = read.csv("./data/LTEE_Newt_Seasonal_OTU_table_97_forprimer.csv")
tomelt = subset(tomelt, select = c(X.OTU.ID, AmphibID, Timepoint, Date,
                                   Substrate.Addition))

# we only consider the timepoints/newts with both metabolite & microbiome data
metabolite = metabolite %>% left_join(tomelt, by = c("NewtID"="AmphibID",
                                                     "Timepoint", "Date")) %>%
  tidyr::drop_na(X.OTU.ID)
otulist = unique(as.character(metabolite$X.OTU.ID))

metabolite = subset(metabolite, select = -c(NewtID, Timepoint,  Date,
                                            Month,  Season, Year,   Season_Year,
                                            Month_Year, Temp,   DO, pH, count,
                                            X.OTU.ID,Substrate.Addition))

microbiome = read.csv("./data/LTEE_Newt_Seasonal_2.csv")
microbiome = microbiome[,as.character(colnames(microbiome)) %in% otulist]
microbiome = t(microbiome)
```

Now we use `vegan` to calculate distance matrices:

```
metabolitedist = vegdist(metabolite, method="jaccard",na.rm=TRUE)
microbiomedist = vegdist(microbiome, method="jaccard",na.rm=TRUE)
```

Let's look at the matrices and make sure they look alright:

```
print(head(as.matrix(metabolitedist)))
```

```
##           1         2         3         4         5         6         7
## 1 0.0000000 0.3369353 0.3566246 0.4215055 0.6191391 0.2933827 0.2929794
## 2 0.3369353 0.0000000 0.1577347 0.4480913 0.7122396 0.1143200 0.3875437
## 3 0.3566246 0.1577347 0.0000000 0.4530109 0.7320797 0.1352044 0.5191555
## 4 0.4215055 0.4480913 0.4530109 0.0000000 0.7493172 0.3381227 0.4919888
## 5 0.6191391 0.7122396 0.7320797 0.7493172 0.0000000 0.7326608 0.5665329
## 6 0.2933827 0.1143200 0.1352044 0.3381227 0.7326608 0.0000000 0.2650762
##           8         9        10        11        12        13        14
## 1 0.6217511 0.6350135 0.2843151 0.5921728 0.4617450 0.6095371 0.5020715
## 2 0.5233981 0.5986355 0.5118284 0.4941138 0.4621888 0.4900141 0.4213766
## 3 0.5137953 0.6051370 0.5258773 0.4799072 0.4480596 0.4761163 0.4251961
## 4 0.4108157 0.3970939 0.3370577 0.3695799 0.2386632 0.3448749 0.2593995
## 5 0.7774723 0.7525886 0.6885955 0.8362129 0.6820028 0.8357843 0.7997769
## 6 0.5741783 0.4809261 0.2318233 0.4894771 0.2811280 0.5308464 0.4713818
##          15        16        17        18        19        20        21
## 1 0.6439978 0.6227019 0.7036857 0.5096826 0.7206789 0.4584556 0.5701677
## 2 0.6264663 0.6079234 0.4895921 0.4818138 0.5365322 0.4740536 0.1391312
## 3 0.6278309 0.5985208 0.4697961 0.4912579 0.5094025 0.4192541 0.1373124
## 4 0.4829098 0.4443365 0.4508240 0.2105587 0.6944517 0.2644328 0.3443421
## 5 0.8349661 0.8167101 0.7897247 0.7363193 0.8586363 0.8981260 0.8943483
## 6 0.5973246 0.4450159 0.5700926 0.3790267 0.6023465 0.2354593 0.2508996
##          22        23        24        25        26        27        28
## 1 0.5416051 0.3994730 0.7586939 0.6228509 0.5530499 0.4223080 0.4565701
## 2 0.1003134 0.7854723 0.5883719 0.8027490 0.6716519 0.5115047 0.4524477
## 3 0.1043098 0.8182882 0.6275298 0.8410359 0.7134729 0.5966466 0.4762411
## 4 0.3406019 0.4423018 0.6742559 0.8095471 0.6307975 0.4297127 0.2529565
## 5 0.8755280 0.6141941 0.5650808 0.4079994 0.6353943 0.5395066 0.8960804
## 6 0.1735785 0.5094079 0.7160446 0.5559860 0.8059088 0.4465250 0.2081633
##          29         30        31        32        33        34        35
## 1 0.6475257 0.50958057 0.2766273 0.6810673 0.7072170 0.3391880 0.7577504
## 2 0.6647271 0.51290150 0.7699380 0.5522503 0.8389955 0.6896399 0.6922984
## 3 0.7251090 0.61213578 0.8434538 0.6246396 0.8993792 0.7365920 0.8244839
## 4 0.6407025 0.29015880 0.2538334 0.6695986 0.8351301 0.4666355 0.7180434
## 5 0.7481569 0.71336808 0.3736316 0.5551170 0.6485932 0.7093265 0.6895183
## 6 0.7779190 0.07213066 0.1680146 0.8214784 0.6481709 0.4220486 0.8613443
##          36        37        38        39        40        41        42
## 1 0.7153251 0.6862135 0.9049856 0.7531032 0.7063984 0.3716024 0.8553890
## 2 0.6240493 0.8890428 0.7989238 0.5304172 0.3677354 0.4646872 0.7332470
## 3 0.6867210 0.9208300 0.8608622 0.5764879 0.6602902 0.5824940 0.8156625
## 4 0.6508154 0.8930944 0.9004050 0.9297707 0.6464863 0.8693815 0.8826077
## 5 0.7075556 0.6112354 0.7758779 0.8214847 0.7612311 0.6873066 0.6188682
## 6 0.8926685 0.6404566 0.9322662 0.8076461 0.4198473 0.4494344 0.8315104
##          43        44        45        46        47        48        49
## 1 0.8716278 0.3190953 0.2157765 0.2654601 0.5109566 0.5867342 0.4196548
## 2 0.7681148 0.4733904 0.4622806 0.3722243 0.4132514 0.3186512 0.5533518
## 3 0.8409031 0.6945828 0.5312342 0.2452584 0.3780754 0.3147359 0.6170357
## 4 0.9335282 0.5895019 0.5786998 0.8438586 0.2388210 0.4562188 0.7309089
## 5 0.7497465 0.8063722 0.6388772 0.8640652 0.7338322 0.7732032 0.8985937
## 6 0.9567793 0.2556818 0.4589332 0.6602239 0.3597959 0.4228582 0.3928419
##          50        51         52        53        54        55        56
## 1 0.8599211 0.5263543 0.01184578 0.8607612 0.7490210 0.8137608 0.7714707
## 2 0.7345463 0.6794622 0.87209481 0.7701371 0.8140231 0.4641440 0.7440726
## 3 0.8545526 0.7894025 0.94042948 0.8203616 0.8486297 0.4447211 0.8330311
```

```
## 4 0.8123551 0.5449438 0.33657563 0.8904396 0.7892203 0.9344061 0.8838968
## 5 0.7083324 0.8514257 0.81905134 0.3606918 0.4388059 0.7353049 0.3979399
## 6 0.8552791 0.3274969 0.87828533 0.8447067 0.8059925 0.7047396 0.8489845
##           57        58        59         60        61        62        63
## 1 0.71959014 0.7578848 0.5626213 0.03330521 0.5562438 0.6662092 0.6500666
## 2 0.73346147 0.7832235 0.6047298 0.27910170 0.4337211 0.7019574 0.5314541
## 3 0.80530391 0.8161855 0.5883843 0.29190076 0.3544261 0.8641943 0.4821566
## 4 0.85613898 0.8801624 0.1637208 0.44329997 0.3691692 0.4455714 0.3568746
## 5 0.07548936 0.4849321 0.8461465 0.74006481 0.7185173 0.9140886 0.8795333
## 6 0.78132045 0.7906862 0.4248630 0.20850930 0.4736805 0.7507244 0.5191678
##          64        65        66        67        68        69        70
## 1 0.2043280 0.3607047 0.6129099 0.3674013 0.3739057 0.6521206 0.3751897
## 2 0.7734247 0.7302428 0.6143519 0.7683890 0.8042094 0.6278717 0.7318710
## 3 0.8191843 0.8068571 0.7356077 0.8339544 0.8206218 0.3633686 0.8004079
## 4 0.2617621 0.4258145 0.5559029 0.5067113 0.4671027 0.4748857 0.4070513
## 5 0.7735849 0.8797197 0.6926808 0.8352893 0.8218838 0.8974354 0.8018121
## 6 0.2312833 0.4745736 0.8810850 0.5937765 0.6685493 0.6993721 0.5396504
##          71        72        73        74        75
## 1 0.5021192 0.4210096 0.5204485 0.5778598 0.4916122
## 2 0.6610145 0.5722289 0.7126395 0.8476242 0.5887875
## 3 0.7573005 0.5912878 0.5237349 0.8907594 0.7051953
## 4 0.2785255 0.3036059 0.1184176 0.6754676 0.1241934
## 5 0.7595414 0.4354793 0.8904788 0.8915279 0.7072694
## 6 0.6768206 0.6985262 0.5399754 0.7451892 0.7137758
```

```
print(head(as.matrix(microbiomedist)))
```

```
##            LTEE.5..1 LTEE.5..2 LTEE.5..8 LTEE2.5.13 LTEE.5..4 LTEE.5..5
## LTEE.5..1  0.0000000 0.5551293 0.7201271  0.6276087 0.6486486 0.5010596
## LTEE.5..2  0.5551293 0.0000000 0.7085857  0.6819979 0.4597408 0.6561008
## LTEE.5..8  0.7201271 0.7085857 0.0000000  0.4042700 0.7752861 0.7046054
## LTEE2.5.13 0.6276087 0.6819979 0.4042700  0.0000000 0.6365453 0.6266338
## LTEE.5..4  0.6486486 0.4597408 0.7752861  0.6365453 0.0000000 0.6345552
## LTEE.5..5  0.5010596 0.6561008 0.7046054  0.6266338 0.6345552 0.0000000
##             LTEE5.11 LTEE.5..6  LTEE5.12 LTEE.5..3   LTEE5.9 LTEE.6..1
## LTEE.5..1  0.9687972 0.6162802 0.6172039 0.9732514 0.8914585 0.6920735
## LTEE.5..2  0.9508719 0.7171547 0.6609719 0.7531708 0.8726919 0.7699094
## LTEE.5..8  0.5695612 0.7867378 0.3700998 0.9446486 0.4173761 0.7998924
## LTEE2.5.13 0.6900056 0.6455560 0.2281420 0.9537673 0.5749036 0.8478805
## LTEE.5..4  0.9534653 0.6501897 0.6321077 0.6692669 0.8969609 0.8760561
## LTEE.5..5  0.9503782 0.4106105 0.6137336 0.9712299 0.8747914 0.7815638
##            LTEE.6..2 LTEE.6..8 LTEE2.6.13 LTEE.6..4 LTEE.6..5 LTEE.6..6
## LTEE.5..1  0.7062812 0.6712790  0.9957753 0.6151238 0.7516680 0.5963554
## LTEE.5..2  0.3127273 0.6986762  0.9692737 0.4262651 0.5937348 0.7115978
## LTEE.5..8  0.7221010 0.2764769  0.6594555 0.6853736 0.7285163 0.7465964
## LTEE2.5.13 0.6944582 0.2451363  0.7292128 0.6476719 0.8021973 0.6290037
## LTEE.5..4  0.3607947 0.6636561  0.9735785 0.3285303 0.6922799 0.6272839
## LTEE.5..5  0.7103373 0.6489320  0.9951490 0.5637165 0.6923094 0.3465891
##             LTEE6.12 LTEE.6..3   LTEE6.9   LTEE7.2 LTEE.7..1   LTEE7.4
## LTEE.5..1  0.8808490 0.9414127 0.8554062 0.6498126 0.2414650 0.7095052
## LTEE.5..2  0.8583351 0.7073481 0.8214257 0.6710050 0.5558848 0.4623134
## LTEE.5..8  0.4012790 0.9063826 0.3674848 0.7948553 0.7271211 0.7883346
## LTEE2.5.13 0.5406235 0.9160094 0.5137732 0.6466622 0.6610956 0.6879906
## LTEE.5..4  0.8739832 0.6262109 0.8629235 0.5940076 0.6996390 0.1906663
## LTEE.5..5  0.8605780 0.9365603 0.8544568 0.4500267 0.5840881 0.7018800
##              LTEE7.5 LTEE.7..6   LTEE7.3 LTEE.8..1 LTEE.8..2 LTEE.8..8
## LTEE.5..1  0.6219350 0.5955736 0.6199676 0.3580568 0.6510373 0.6629475
## LTEE.5..2  0.5812480 0.7092754 0.6174676 0.5586481 0.7054438 0.7292406
## LTEE.5..8  0.7712645 0.7447246 0.7749498 0.7272328 0.7978776 0.4957726
## LTEE2.5.13 0.6235702 0.6327204 0.6273164 0.6535738 0.6562254 0.3729803
## LTEE.5..4  0.4549814 0.6149915 0.5571421 0.6927221 0.6243537 0.6443213
## LTEE.5..5  0.4155603 0.3125309 0.4297163 0.5631116 0.4478698 0.5597934
##            LTEE2.8.13 LTEE.8..4 LTEE.8..5  LTEE8.11 LTEE.8..6  LTEE8.12
## LTEE.5..1   0.6678304 0.6637792 0.6310746 0.7375661 0.6299428 0.6701518
## LTEE.5..2   0.7344258 0.7570718 0.6217713 0.7361081 0.7098209 0.7526345
## LTEE.5..8   0.5987292 0.8229803 0.7753378 0.2470986 0.7616628 0.8242449
## LTEE2.5.13  0.3861543 0.6879312 0.6305575 0.4745674 0.6326882 0.6799882
## LTEE.5..4   0.6536363 0.6674631 0.5214266 0.8261010 0.6092795 0.6699688
## LTEE.5..5   0.4681615 0.4618649 0.4188861 0.7191096 0.3887099 0.4714719
##            LTEE.8..3   LTEE8.9 LTEE.9..1 LTEE.9..2 LTEE.9..8 LTEE2.9.13
## LTEE.5..1  0.9321157 0.9896702 0.4757700 0.6624233 0.6351978  0.6722828
## LTEE.5..2  0.6905971 0.9614668 0.5837423 0.6804687 0.7112829  0.7480844
## LTEE.5..8  0.9036746 0.6377302 0.7326837 0.8031119 0.4157334  0.6852544
## LTEE2.5.13 0.9127990 0.7230295 0.6261459 0.6591456 0.2862546  0.4976429
## LTEE.5..4  0.6047631 0.9663898 0.6364812 0.5975094 0.6151569  0.6664827
## LTEE.5..5  0.9278122 0.9808111 0.3220991 0.4614977 0.5886704  0.4750887
##            LTEE.9..4 LTEE.9..5  LTEE9.11 LTEE2.9.12 LTEE.9..3   LTEE9.9
## LTEE.5..1  0.5810398 0.8756857 0.8656144  0.6680140 0.7411009 0.7701442
## LTEE.5..2  0.6906858 0.6590215 0.8272641  0.7575777 0.3873658 0.7362733
## LTEE.5..8  0.7613190 0.8461615 0.4184550  0.8262436 0.7495149 0.1965735
```

```
## LTEE2.5.13 0.6325592 0.8864143 0.6290685  0.6801384 0.7203248 0.3980556
## LTEE.5..4  0.6389130 0.5649958 0.9019934  0.6739826 0.3433778 0.7880054
## LTEE.5..5  0.3586144 0.8568050 0.8653703  0.4693339 0.7435281 0.7709000
##            LTEE.13..1 LTEE.13..2 LTEE.13..8 LTEE.13..4 LTEE.13..5 LTEE13.11
## LTEE.5..1   0.1974142  0.8358556  0.5318314  0.8817337  0.8993681 0.9544275
## LTEE.5..2   0.5580031  0.5195395  0.6127057  0.9041924  0.6812487 0.9329409
## LTEE.5..8   0.7259188  0.8041730  0.3744850  0.8722755  0.8779912 0.5450532
## LTEE2.5.13  0.6482707  0.8516671  0.2490037  0.9014319  0.8924323 0.6676774
## LTEE.5..4   0.6879906  0.5759533  0.6212143  0.9150767  0.5739575 0.9426092
## LTEE.5..5   0.5615079  0.8368595  0.5474581  0.8180151  0.8969190 0.9393025
##            LTEE.13..6 LTEE.13..3  LTEE13.9 LTEE.16..1 LTEE.16..2 LTEE.16..8
## LTEE.5..1   0.6049308  0.9869526 0.7779182  0.4439556  0.9730333  0.7647222
## LTEE.5..2   0.5145726  0.7798721 0.7513724  0.6606318  0.7409369  0.7330435
## LTEE.5..8   0.7064832  0.9619315 0.3032005  0.7259747  0.9501502  0.2987710
## LTEE2.5.13  0.7786645  0.9715036 0.5024915  0.6279334  0.9555760  0.3383469
## LTEE.5..4   0.7107673  0.6992891 0.8571879  0.6487116  0.7321019  0.7378958
## LTEE.5..5   0.7199294  0.9865278 0.8119866  0.5129349  0.9722326  0.7640114
##            LTEE.16..4 LTEE.16..5 LTEE16.11 LTEE.16..6 LTEE2.16.12 LTEE.16..3
## LTEE.5..1   0.6574385  0.6512569 0.9681918  0.6328493   0.6620531  0.6521032
## LTEE.5..2   0.7590398  0.5590778 0.9459145  0.5885330   0.7363559  0.5236471
## LTEE.5..8   0.8099879  0.7839906 0.5849172  0.7702746   0.7256948  0.7841945
## LTEE2.5.13  0.6773392  0.6491208 0.6759805  0.6256576   0.5488391  0.6466937
## LTEE.5..4   0.6570344  0.3333812 0.9441107  0.4942496   0.6610338  0.4366577
## LTEE.5..5   0.4392659  0.4835412 0.9588569  0.4182393   0.4653243  0.4546109
##             LTEE16.9  LTEE17.8 LTEE2.17.13  LTEE17.5 LTEE2.17.11  LTEE17.6
## LTEE.5..1  0.5798585 0.7956060   0.6688394 0.7348123   0.8762957 0.6584012
## LTEE.5..2  0.6635637 0.7711083   0.7437730 0.4885206   0.8626113 0.6446381
## LTEE.5..8  0.6364171 0.2371892   0.6825966 0.7078376   0.5519187 0.7939782
## LTEE2.5.13 0.4798050 0.3812899   0.4954604 0.7854166   0.6237335 0.6489634
## LTEE.5..4  0.6398068 0.7720710   0.6603534 0.4063745   0.9317813 0.5552373
## LTEE.5..5  0.3791186 0.7888406   0.4704238 0.6974488   0.8731957 0.4538281
##            LTEE2.17.12  LTEE17.3 LTEE2.17.9
## LTEE.5..1    0.6660227 0.8975689  0.6196392
## LTEE.5..2    0.7337074 0.6398068  0.7026930
## LTEE.5..8    0.5761281 0.8680718  0.6341693
## LTEE2.5.13   0.3511954 0.8766439  0.4357301
## LTEE.5..4    0.6493724 0.5307901  0.6361606
## LTEE.5..5    0.4854024 0.8964572  0.4187137
```

That's what we expect. Now let's perform a Mantel test.

But first, let's put this whole process into a function, so that we can do it again easily, and also subset if we want:

```
processMetMic <- function(substratefilter="") {
  metabolite = read.csv("./data/metabolite.csv")
  tomelt = read.csv("./data/LTEE_Newt_Seasonal_OTU_table_97_forprimer.csv")
  tomelt = subset(tomelt, select = c(X.OTU.ID, AmphibID, Timepoint, Date,
                                  Substrate.Addition))

  # we only consider the timepoints/newts with both metabolite & microbiome data
  if (substratefilter == "") {
    metabolite = metabolite %>% left_join(tomelt, by = c("NewtID"="AmphibID",
                                                          "Timepoint", "Date")) %>%
      tidyr::drop_na(X.OTU.ID)
  } else {
    metabolite = metabolite %>% left_join(tomelt, by = c("NewtID"="AmphibID",
                                                        "Timepoint", "Date")) %>%
    tidyr::drop_na(X.OTU.ID) %>%
    dplyr::filter(as.character(Substrate.Addition) == substratefilter)
    # choose from "Pre substrate addition", "Post substrate addition 1",
    # and "Post substrate addition 2"
  }
  otulist = unique(as.character(metabolite$X.OTU.ID))

  metabolite = subset(metabolite, select = -c(NewtID,   Timepoint,  Date,
                                              Month,    Season, Year,   Season_Year,
                                              Month_Year,   Temp,   DO, pH, count,
                                              X.OTU.ID,Substrate.Addition))

  microbiome = read.csv("./data/LTEE_Newt_Seasonal_2.csv")
  microbiome = microbiome[,as.character(colnames(microbiome)) %in% otulist]
  microbiome = t(microbiome)

  metabolitedist = vegdist(metabolite, method="jaccard",na.rm=TRUE)
  microbiomedist = vegdist(microbiome, method="jaccard",na.rm=TRUE)
  return(list("microbiomedist" = microbiomedist, "metabolitedist" = metabolitedist))
}
```

Let's start with a Mantel test on the full dataset:

```
mantel.rtest(metabolitedist, microbiomedist, nrepet = 999)
```

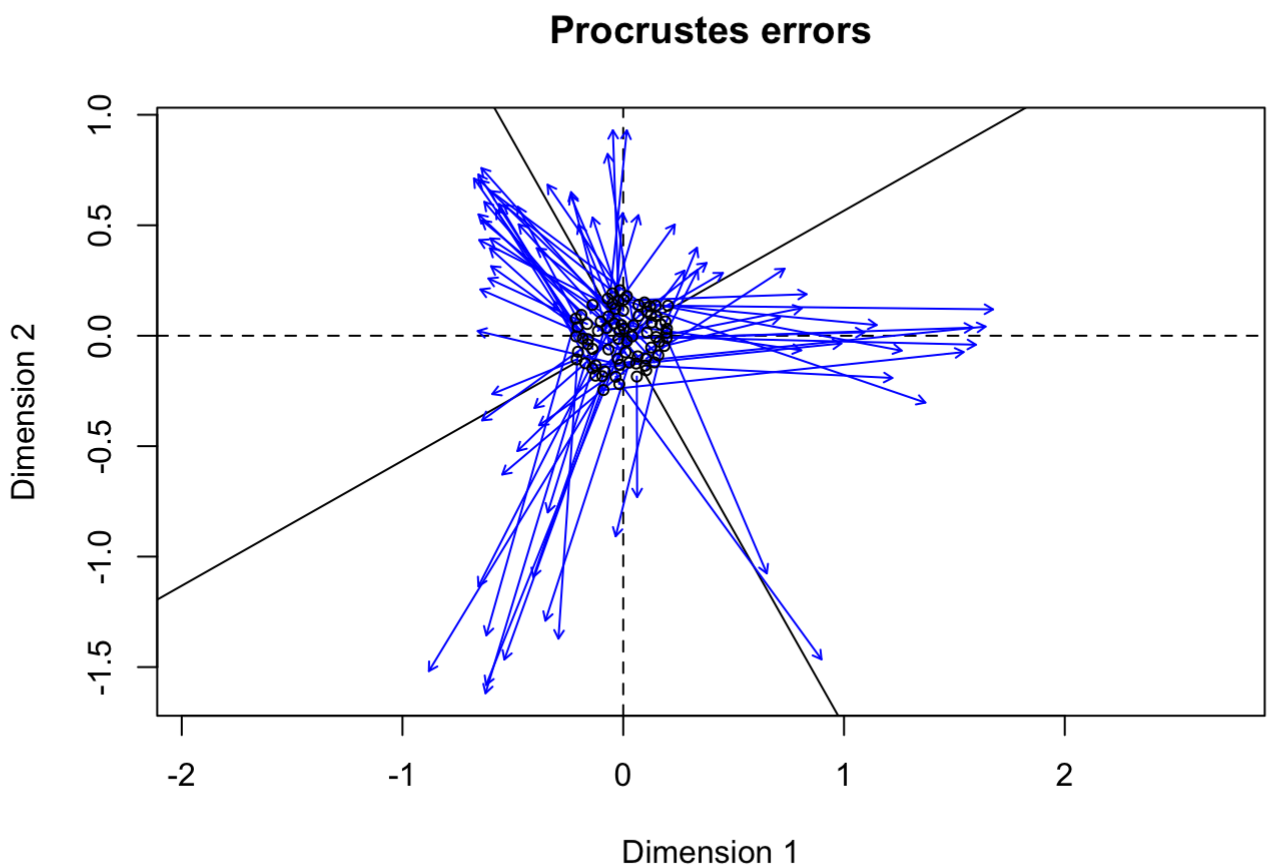
```
## Monte-Carlo test
## Call: mantelnoneuclid(m1 = m1, m2 = m2, nrepet = nrepet)
##
## Observation: 0.03668781
##
## Based on 999 replicates
## Simulated p-value: 0.134
## Alternative hypothesis: greater
##
##     Std.Obs Expectation    Variance
## 1.151804502 0.002656944 0.000872948
```

We can see that on the full dataset, the Mantel test is marginally significant (some stochastic samples end up with $p < 0.05$, some are slightly over 0.05). Let's try a Procrustes rotation and see whether it is significant:

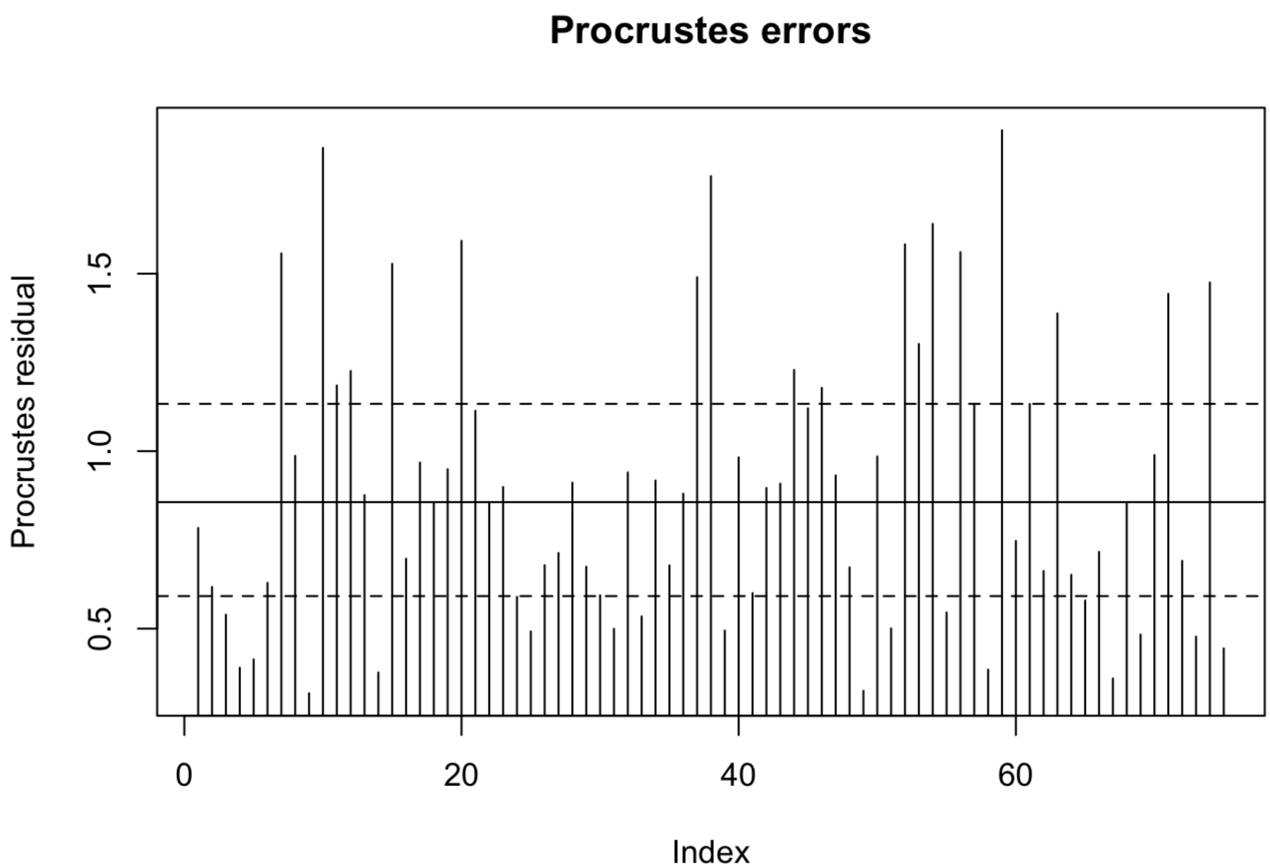
```
# Do a MDS on the distance matrices
microbiomeMDS <- monoMDS(microbiomedist)
metaboliteMDS <- monoMDS(metabolitedist)
# Perform Procrustes
vare.proc <- procrustes(microbiomeMDS, metaboliteMDS)
summary(vare.proc)
```

```
##
## Call:
## procrustes(X = microbiomeMDS, Y = metaboliteMDS)
##
## Number of objects: 75    Number of dimensions: 2
##
## Procrustes sum of squares:
##  72.93086
## Procrustes root mean squared error:
##  0.9861092
## Quantiles of Procrustes errors:
##       Min        1Q    Median        3Q       Max
## 0.3180248 0.5917816 0.8564700 1.1333472 1.9040722
##
## Rotation matrix:
##            [,1]       [,2]
## [1,] -0.8703015 -0.4925193
## [2,] -0.4925193  0.8703015
##
## Translation of averages:
##              [,1]         [,2]
## [1,] 8.696758e-18 6.391683e-18
##
## Scaling of target:
## [1] 0.1660981
```

```
plot(vare.proc)
```




```
plot(vare.proc, kind=2)
```




```
residuals(vare.proc)
```

```
##   LTEE.5..1   LTEE.5..2   LTEE.5..8  LTEE2.5.13   LTEE.5..4   LTEE.5..5 
##   0.7836980   0.6175752   0.5393723   0.3901111   0.4135309   0.6290963 
##    LTEE5.11   LTEE.5..6    LTEE5.12   LTEE.5..3     LTEE5.9   LTEE.6..1 
##   1.5572179   0.9867366   0.3180248   1.8545483   1.1852174   1.2261082 
##   LTEE.6..2   LTEE.6..8  LTEE2.6.13   LTEE.6..4   LTEE.6..5   LTEE.6..6 
##   0.8761916   0.3766355   1.5278233   0.6970496   0.9679641   0.8564700 
##    LTEE6.12   LTEE.6..3     LTEE6.9     LTEE7.2   LTEE.7..1     LTEE7.4 
##   0.9492929   1.5927474   1.1137795   0.8542958   0.8990507   0.5896747 
##     LTEE7.5   LTEE.7..6     LTEE7.3   LTEE.8..1   LTEE.8..2   LTEE.8..8 
##   0.4923875   0.6788085   0.7131163   0.9113533   0.6746255   0.5938885 
##  LTEE2.8.13   LTEE.8..4   LTEE.8..5    LTEE8.11   LTEE.8..6    LTEE8.12 
##   0.4997364   0.9402794   0.5345694   0.9172633   0.6783808   0.8802037 
##   LTEE.8..3     LTEE8.9   LTEE.9..1   LTEE.9..2   LTEE.9..8  LTEE2.9.13 
##   1.4899771   1.7748460   0.4944883   0.9825257   0.6001638   0.8962796 
##   LTEE.9..4   LTEE.9..5    LTEE9.11  LTEE2.9.12   LTEE.9..3     LTEE9.9 
##   0.9086530   1.2288995   1.1208235   1.1783640   0.9318287   0.6725033 
##  LTEE.13..1  LTEE.13..2  LTEE.13..8  LTEE.13..4  LTEE.13..5    LTEE13.11 
##   0.3250149   0.9851996   0.5006184   1.5828658   1.3019107   1.6404114 
##  LTEE.13..6  LTEE.13..3    LTEE13.9  LTEE.16..1  LTEE.16..2  LTEE.16..8 
##   0.5457525   1.5607274   1.1336264   0.3844944   1.9040722   0.7471982 
##  LTEE.16..4  LTEE.16..5    LTEE16.11  LTEE.16..6 LTEE2.16.12  LTEE.16..3 
##   1.1330680   0.6624180   1.3882464   0.6517171   0.5793223   0.7163242 
##    LTEE16.9     LTEE17.8 LTEE2.17.13    LTEE17.5 LTEE2.17.11    LTEE17.6 
##   0.3597648   0.8519174   0.4833393   0.9891515   1.4434316   0.6911506 
## LTEE2.17.12     LTEE17.3  LTEE2.17.9 
##   0.4775290   1.4754960   0.4442248
```

```
print(protest(microbiomeMDS,metaboliteMDS, scores = "sites",
        permutations = how(nperm = 999)))
```

```
## 
## Call:
## protest(X = microbiomeMDS, Y = metaboliteMDS, scores = "sites",      permutations = h
ow(nperm = 999)) 
## 
## Procrustes Sum of Squares (m12 squared):        0.9724 
## Correlation in a symmetric Procrustes rotation: 0.1661 
## Significance:  0.242 
## 
## Permutation: free
## Number of permutations: 999
```

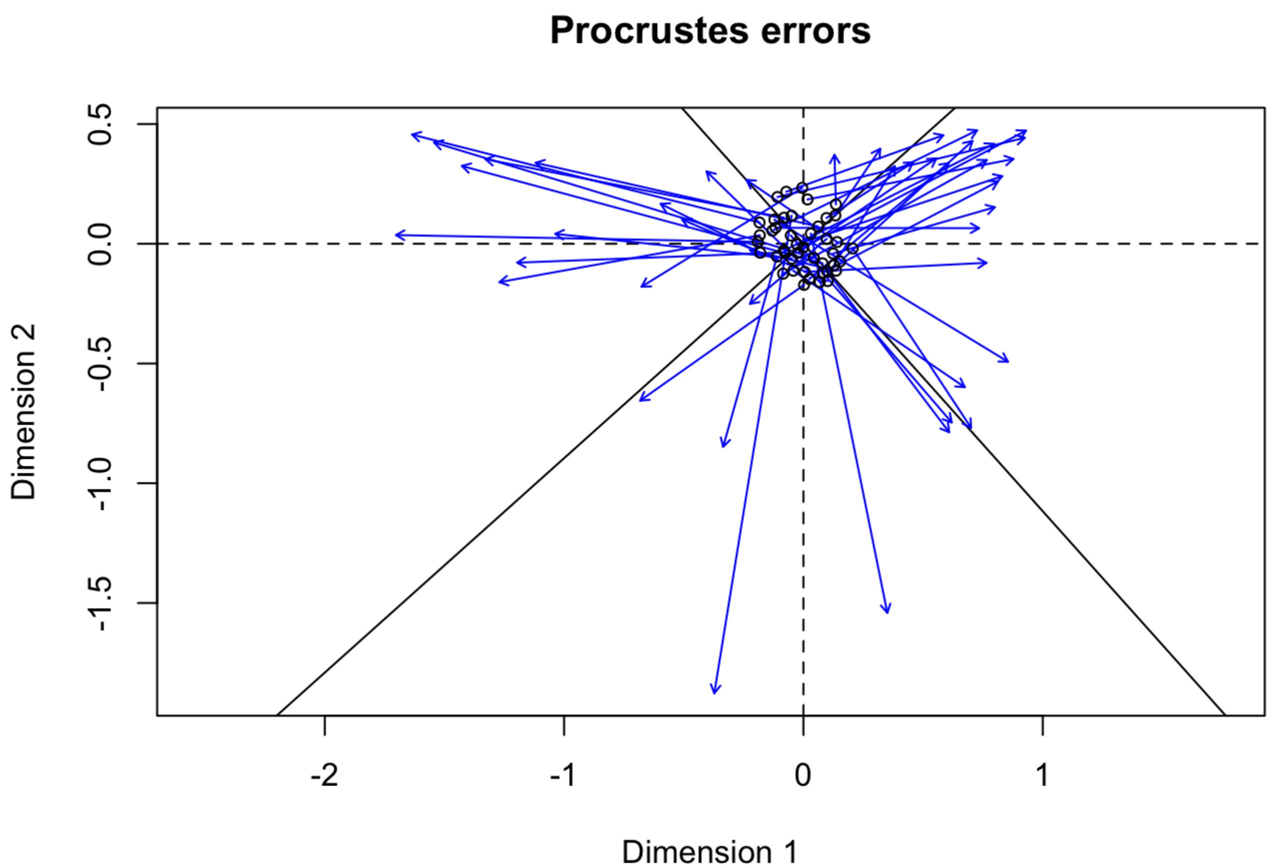
So we can see that the test of significance on the Procrustes rotation is not significant. Let’s do the same thing, but write a function to print everything out first:

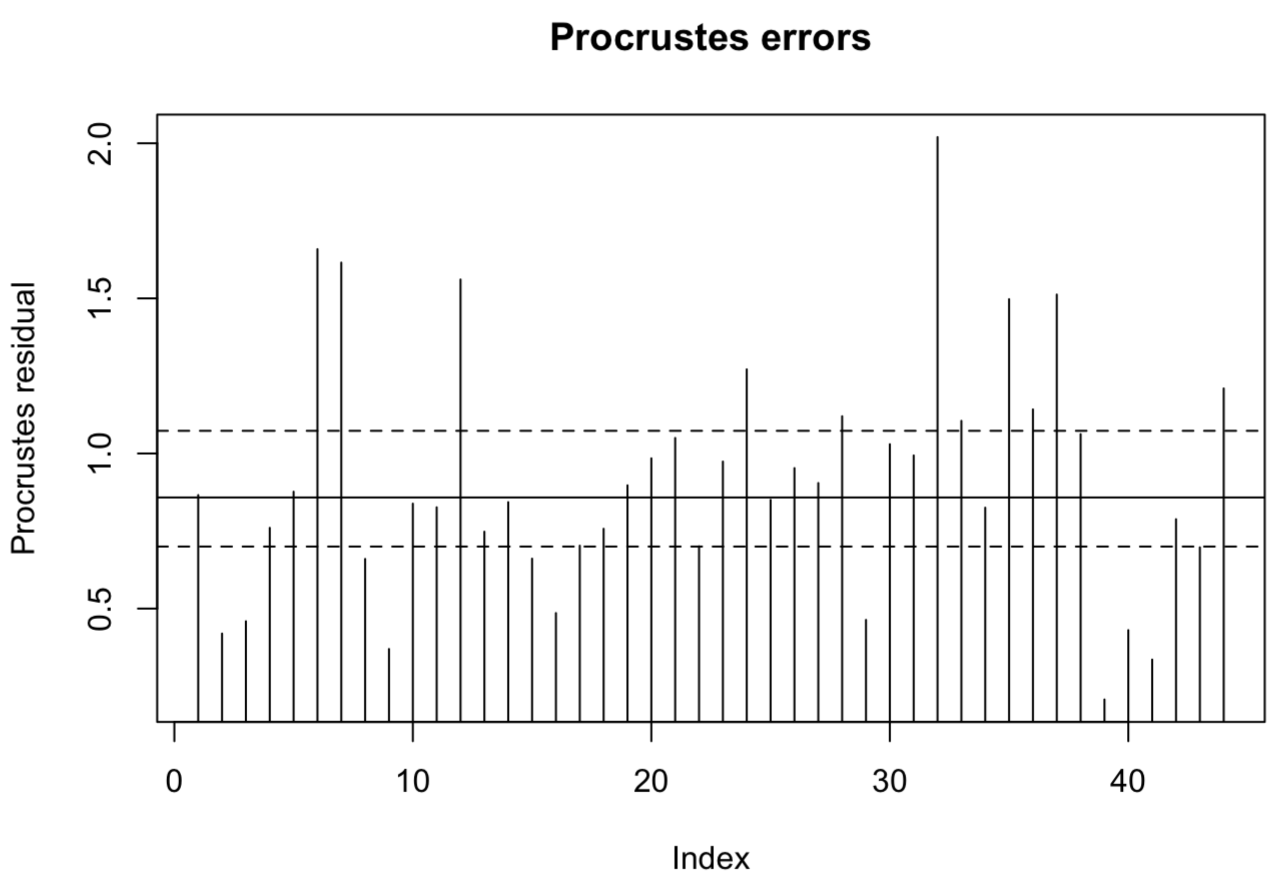
```r
procrustesfunct <- function(metabolitedist, microbiomedist) {
  print(mantel.rtest(metabolitedist, microbiomedist, nrepet = 999))
  # Do a MDS on the distance matrices
  microbiomeMDS <- monoMDS(microbiomedist)
  metaboliteMDS <- monoMDS(metabolitedist)
  # Perform Procrustes
  vare.proc <- procrustes(microbiomeMDS, metaboliteMDS)
  summary(vare.proc)
  plot(vare.proc)
  plot(vare.proc, kind=2)
  residuals(vare.proc)
  print(protest(microbiomeMDS,metaboliteMDS, scores = "sites",
          permutations = how(nperm = 999)))
}
```

Now let's subset to pre-substrate addition:

```r
results = processMetMic(substratefilter = "Pre substrate addition")
microbiomedist = results$microbiomedist
metabolitedist = results$metabolitedist
procrustesfunct(metabolitedist, microbiomedist)
```

```
## Monte-Carlo test
## Call: mantelnoneuclid(m1 = m1, m2 = m2, nrepet = nrepet)
##
## Observation: 0.001074287
##
## Based on 999 replicates
## Simulated p-value: 0.494
## Alternative hypothesis: greater
##
##       Std.Obs   Expectation      Variance
##  0.0347660948 -0.0006170356  0.0023666880
```


Procrustes errors
Dimension 2
Dimension 1
0.5
0.0
-0.5
-1.0
-1.5
-2
-1
0
1

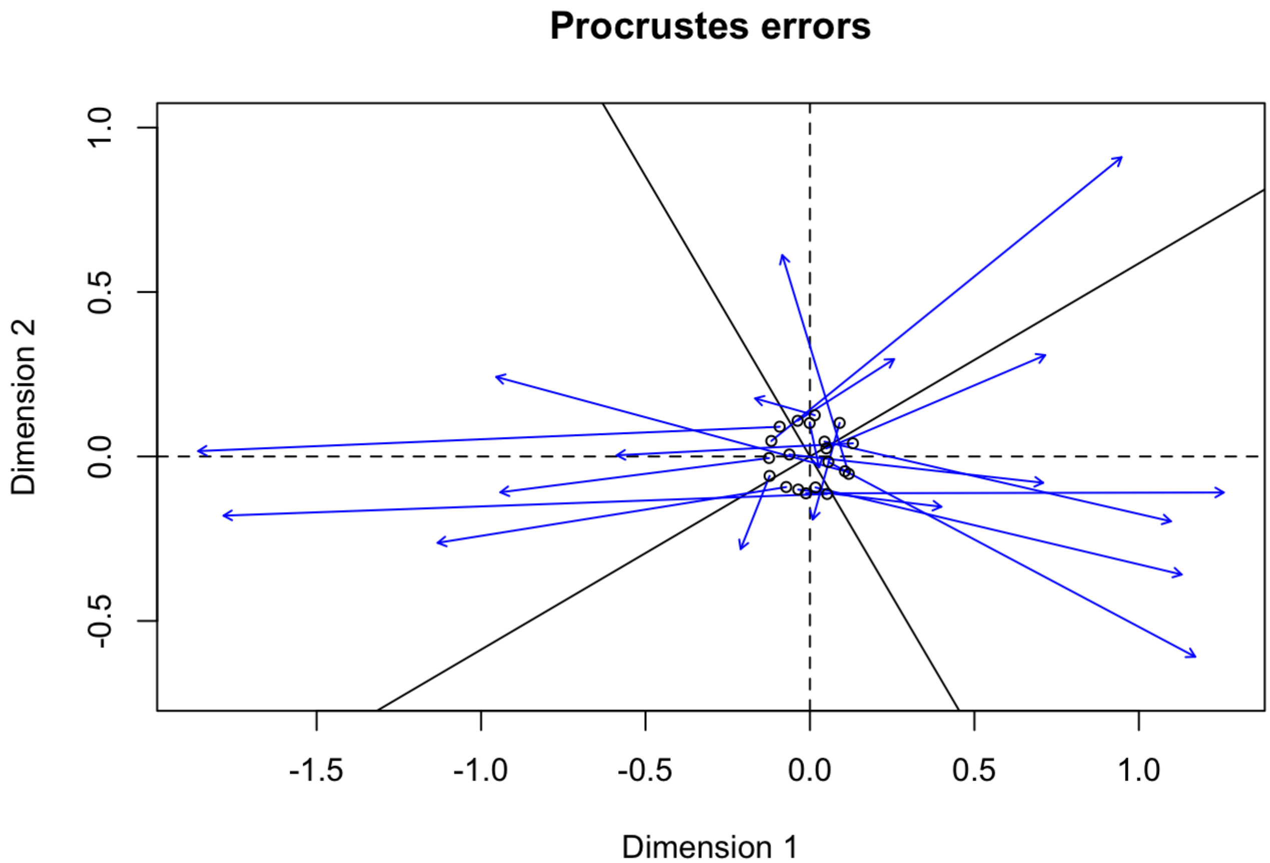

Procrustes errors
Procrustes residual
Index
2.0
1.5
1.0
0.5
0
10
20
30
40

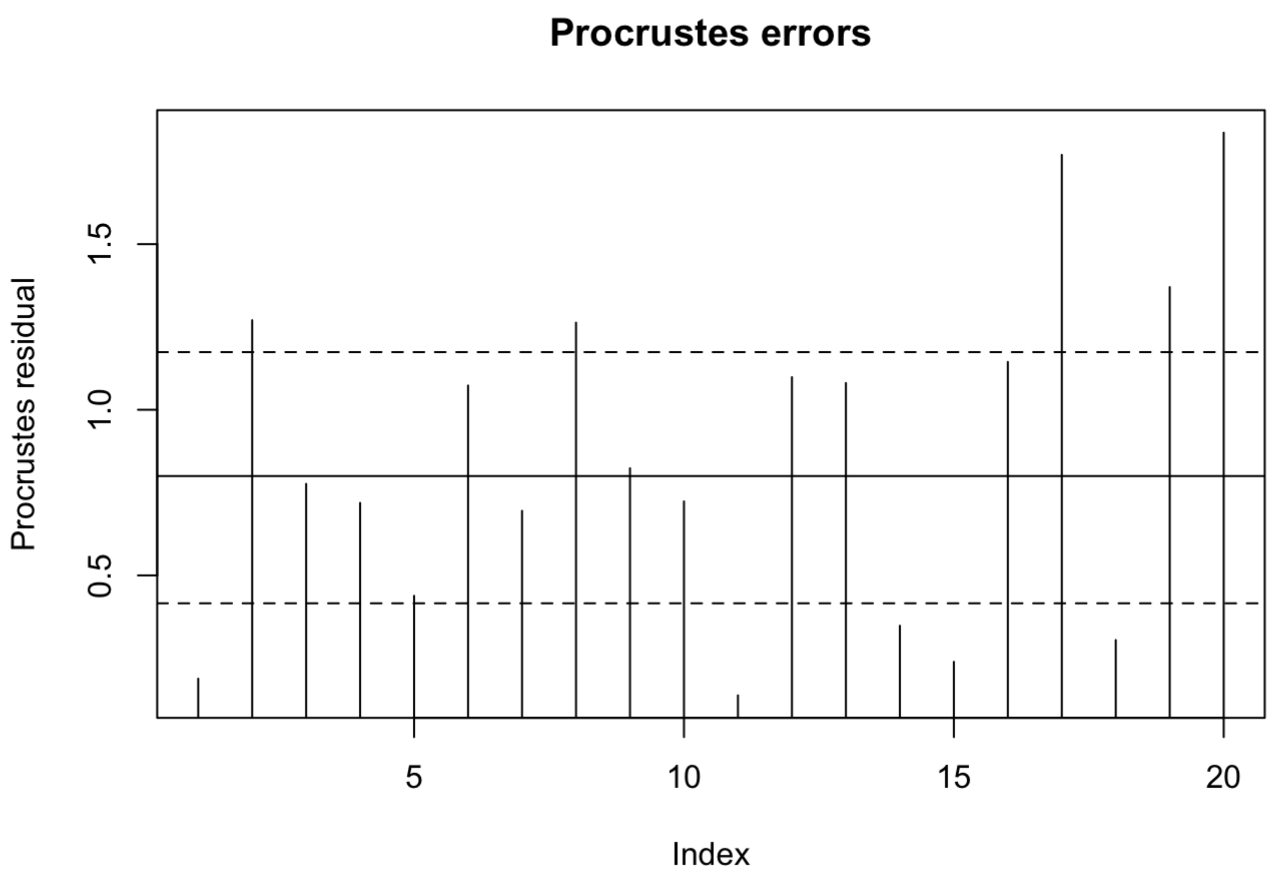
```
##
## Call:
## protest(X = microbiomeMDS, Y = metaboliteMDS, scores = "sites",      permutations = h
ow(nperm = 999))
##
## Procrustes Sum of Squares (m12 squared):        0.9775
## Correlation in a symmetric Procrustes rotation: 0.1501
## Significance:  0.607
##
## Permutation: free
## Number of permutations: 999
```

So none of the tests are significant with only pre-substrate addition data. How about post disturbance 1?

```
results = processMetMic(substratefilter = "Post substrate addition 1")
microbiomedist = results$microbiomedist
metabolitedist = results$metabolitedist
procrustesfunct(metabolitedist, microbiomedist)
```

```
## Monte-Carlo test
## Call: mantelnoneuclid(m1 = m1, m2 = m2, nrepet = nrepet)
##
## Observation: -0.02810831
##
## Based on 999 replicates
## Simulated p-value: 0.638
## Alternative hypothesis: greater
##
##      Std.Obs  Expectation     Variance
## -0.375121476  0.001014124  0.006027141
```


Procrustes errors
Dimension 2
1.0
0.5
0.0
-0.5
-1.5
-1.0
-0.5
0.0
0.5
1.0
Dimension 1

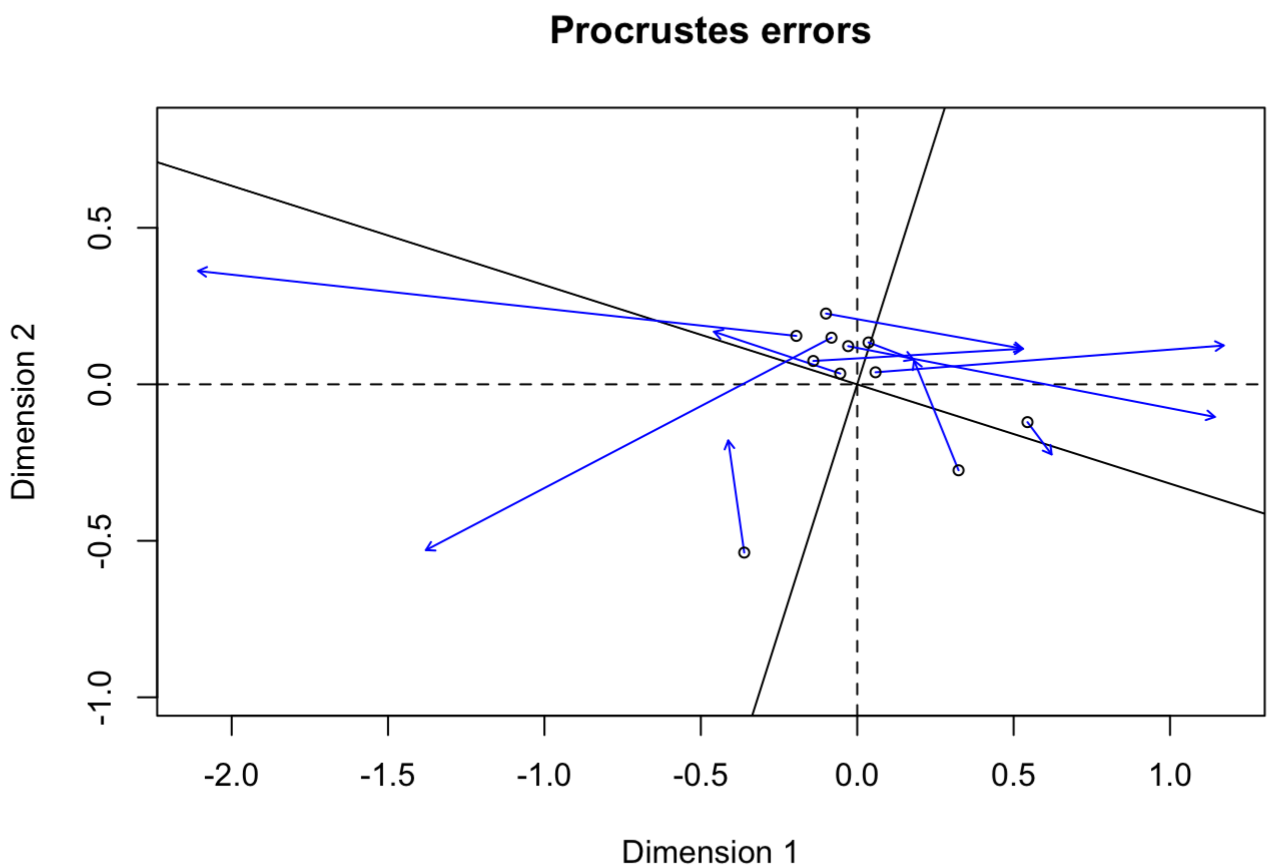

Procrustes errors
Procrustes residual
1.5
1.0
0.5
5
10
15
20
Index

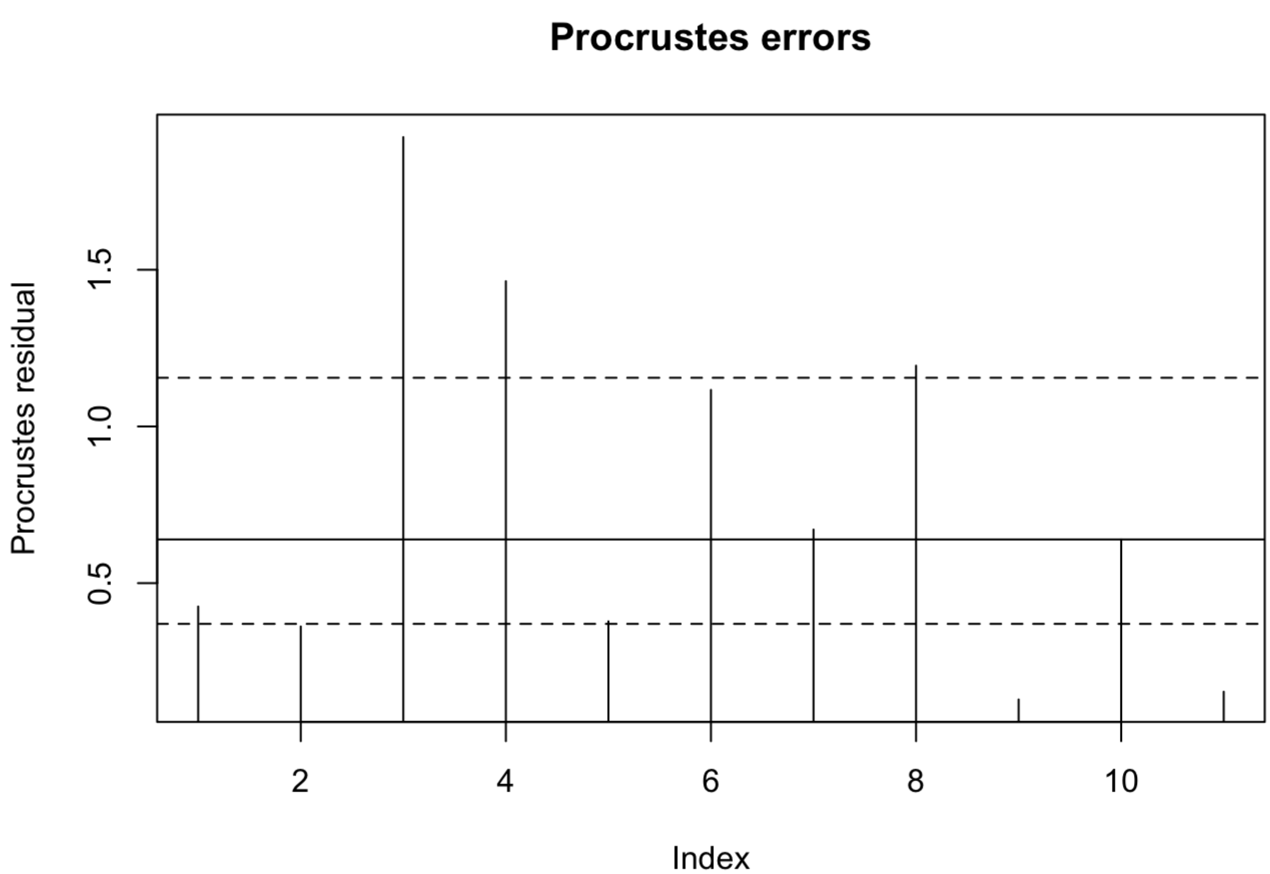
```
##
## Call:
## protest(X = microbiomeMDS, Y = metaboliteMDS, scores = "sites",      permutations = h
ow(nperm = 999))
##
## Procrustes Sum of Squares (m12 squared):        0.9875
## Correlation in a symmetric Procrustes rotation: 0.112
## Significance:  0.93
##
## Permutation: free
## Number of permutations: 999
```

Or post disturbance 2?

```
results = processMetMic(substratefilter = "Post substrate addition 2")
microbiomedist = results$microbiomedist
metabolitedist = results$metabolitedist
procrustesfunct(metabolitedist, microbiomedist)
```

```
## Monte-Carlo test
## Call: mantelnoneuclid(m1 = m1, m2 = m2, nrepet = nrepet)
##
## Observation: -0.1580095
##
## Based on 999 replicates
## Simulated p-value: 0.728
## Alternative hypothesis: greater
##
##      Std.Obs  Expectation     Variance
## -0.702767521 -0.007121717  0.046098269
```


Procrustes errors
Dimension 2
0.5
0.0
-0.5
-1.0
-2.0
-1.5
-1.0
-0.5
0.0
0.5
1.0
Dimension 1

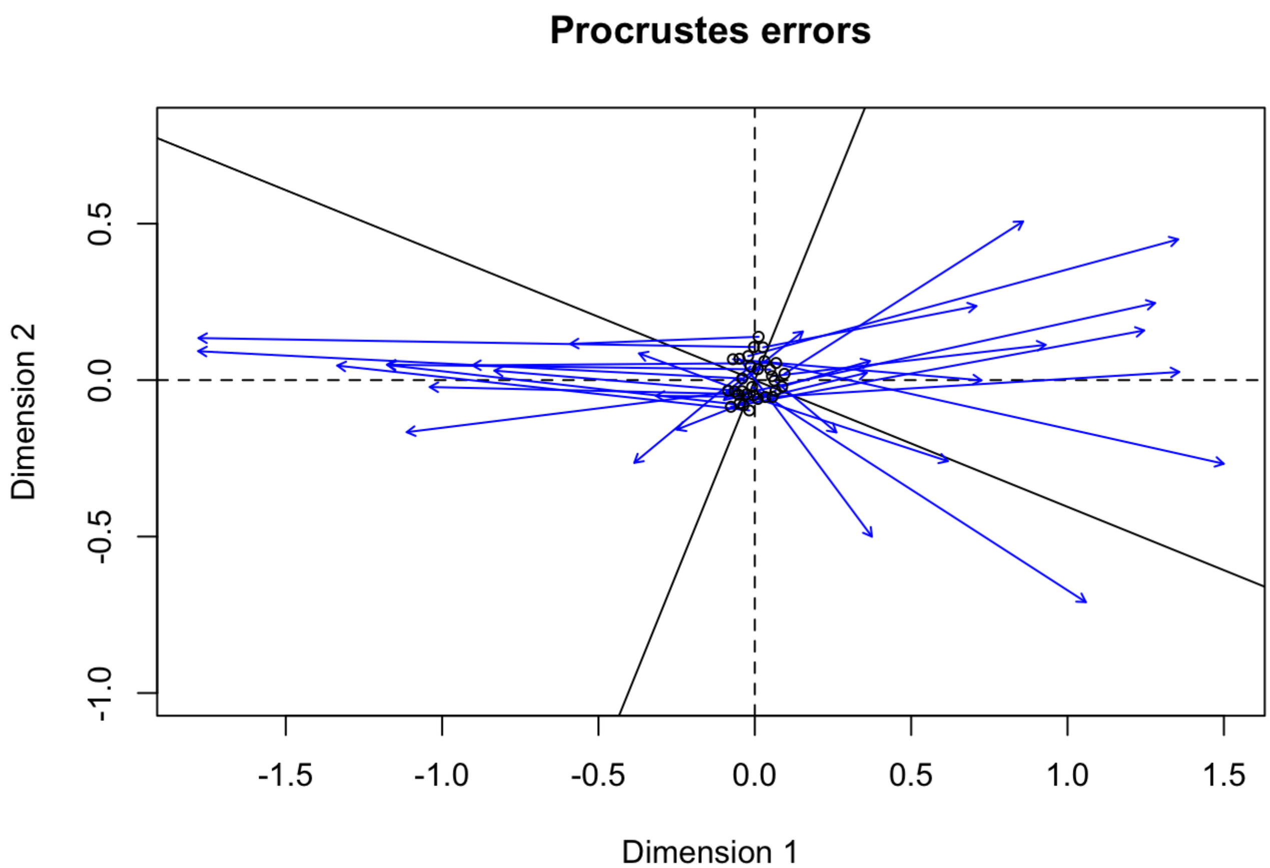

Procrustes errors
Procrustes residual
1.5
1.0
0.5
2
4
6
8
10
Index

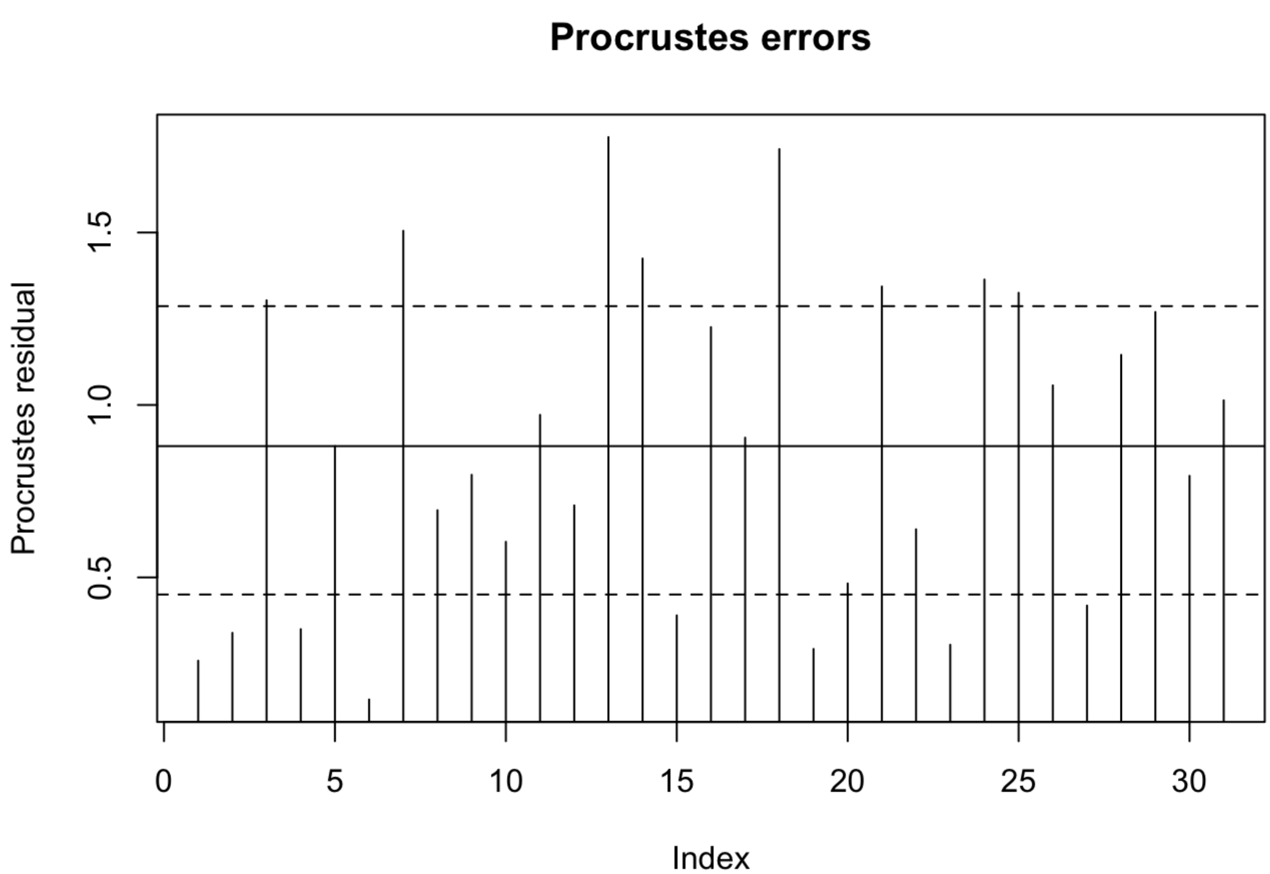
```
##
## Call:
## protest(X = microbiomeMDS, Y = metaboliteMDS, scores = "sites",      permutations = h
ow(nperm = 999))
##
## Procrustes Sum of Squares (m12 squared):        0.8971
## Correlation in a symmetric Procrustes rotation: 0.3208
## Significance:  0.498
##
## Permutation: free
## Number of permutations: 999
```

Or post either disturbance?

```
metabolite = read.csv("./data/metabolite.csv")
tomelt = read.csv("./data/LTEE_Newt_Seasonal_OTU_table_97_forprimer.csv")
tomelt = subset(tomelt, select = c(X.OTU.ID, AmphibID, Timepoint, Date,
                                   Substrate.Addition))

# we only consider the timepoints/newts with both metabolite & microbiome data
metabolite = metabolite %>% left_join(tomelt, by = c("NewtID"="AmphibID",
                                                   "Timepoint", "Date")) %>%
tidyr::drop_na(X.OTU.ID) %>%
dplyr::filter((as.character(Substrate.Addition) == "Post substrate addition 1") |
              (as.character(Substrate.Addition) == "Post substrate addition 2") )
otulist = unique(as.character(metabolite$X.OTU.ID))

metabolite = subset(metabolite, select = -c(NewtID, Timepoint,  Date,
                                            Month,  Season, Year,   Season_Year,
                                            Month_Year, Temp,   DO, pH, count,
                                            X.OTU.ID,Substrate.Addition))

microbiome = read.csv("./data/LTEE_Newt_Seasonal_2.csv")
microbiome = microbiome[,as.character(colnames(microbiome)) %in% otulist]
microbiome = t(microbiome)

metabolitedist = vegdist(metabolite, method="jaccard",na.rm=TRUE)
microbiomedist = vegdist(microbiome, method="jaccard",na.rm=TRUE)
procrustesfunct(metabolitedist, microbiomedist)
```

```
## Monte-Carlo test
## Call: mantelnoneuclid(m1 = m1, m2 = m2, nrepet = nrepet)
##
## Observation: -0.06297983
##
## Based on 999 replicates
## Simulated p-value: 0.909
## Alternative hypothesis: greater
##
##       Std.Obs   Expectation      Variance
## -1.2436056893 -0.0001617191  0.0025515515
```


Procrustes errors
Dimension 1
Dimension 2
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
-1.0
-0.5
0.0
0.5


Procrustes errors
Index
Procrustes residual
0
5
10
15
20
25
30
0.5
1.0
1.5

```
##
## Call:
## protest(X = microbiomeMDS, Y = metaboliteMDS, scores = "sites",      permutations = h
ow(nperm = 999))
##
## Procrustes Sum of Squares (m12 squared):        0.9937
## Correlation in a symmetric Procrustes rotation: 0.0794
## Significance:  0.92
##
## Permutation: free
## Number of permutations: 999
```