MantelProcrustes

Arianna Krinos

9/3/2020

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="bray",na.rm=TRUE)
microbiomedist = vegdist(microbiome, method="bray",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 0.20259904 0.21700741 0.2670301 0.4483718 0.17190891 0.1716320
## 2 0.2025990 0.00000000 0.08561998 0.2887356 0.5530840 0.06062533 0.2403437
## 3 0.2170074 0.08561998 0.00000000 0.2928339 0.5773862 0.07250361 0.3505807
## 4 0.2670301 0.28873557 0.29283392 0.0000000 0.5991265 0.20345830 0.3262501
## 5 0.4483718 0.55308397 0.57738620 0.5991265 0.0000000 0.57810950 0.3952186
## 6 0.1719089 0.06062533 0.07250361 0.2034583 0.5781095 0.00000000 0.1527884
##           8         9        10        11        12        13        14
## 1 0.4511167 0.4652159 0.1657152 0.4206289 0.3001745 0.4383700 0.3351772
## 2 0.3544612 0.4271804 0.3439311 0.3281216 0.3005498 0.3245157 0.2669266
## 3 0.3457096 0.4338326 0.3567391 0.3157091 0.2887093 0.3124361 0.2699994
## 4 0.2585073 0.2477337 0.2026875 0.2266777 0.1355011 0.2083679 0.1490287
## 5 0.6359548 0.6033202 0.5250824 0.7185273 0.5174539 0.7178947 0.6663569
## 6 0.4026999 0.3165916 0.1311087 0.3240448 0.1635538 0.3613281 0.3083712
##          15        16        17        18        19        20         21
## 1 0.4749238 0.4521185 0.5428358 0.3419960 0.5633291 0.2974002 0.39876543
## 2 0.4560983 0.4367026 0.3241456 0.3173615 0.3666171 0.3106620 0.07476682
## 3 0.4575463 0.4270636 0.3070154 0.3256076 0.3417439 0.2652255 0.07371734
## 4 0.3183132 0.2856250 0.2910089 0.1176673 0.5319234 0.1523610 0.20797905
## 5 0.7166882 0.6902029 0.6525165 0.5826783 0.7522899 0.8150895 0.80888798
## 6 0.4258466 0.2861868 0.3986920 0.2338266 0.4309698 0.1334394 0.14344496
##          22        23        24        25        26        27        28
## 1 0.37137066 0.2495884 0.6112061 0.4522756 0.3822177 0.2676745 0.2958153
## 2 0.05280521 0.6467307 0.4168037 0.6704934 0.5056294 0.3436388 0.2923634
## 3 0.05502473 0.6924600 0.4572266 0.7256791 0.5545727 0.4251577 0.3125436
## 4 0.20525629 0.2839457 0.5085867 0.6800329 0.4607043 0.2736523 0.1447912
## 5 0.77861258 0.4432035 0.3938067 0.2562809 0.4656249 0.3694002 0.8117262
## 6 0.09503745 0.3417487 0.5576865 0.3850281 0.6749140 0.2874362 0.1161732
##          29         30         31        32        33        34         35
## 1 0.4787711 0.34190413 0.16051506 0.5163776 0.5470500 0.2042302 0.6099824
## 2 0.4978211 0.34490083 0.62593437 0.3814543 0.7226462 0.5262980 0.5294009
## 3 0.5687616 0.44106316 0.72928671 0.4541643 0.8171562 0.5830199 0.7013804
## 4 0.4713482 0.16969927 0.14536607 0.5033057 0.7169299 0.3043213 0.5601152
## 5 0.5976443 0.55444612 0.22973366 0.3841952 0.4799393 0.5495786 0.5261564
## 6 0.6365527 0.03741471 0.09171177 0.6970414 0.4794770 0.2674662 0.7564572
##          36        37        38        39        40        41        42
## 1 0.5568141 0.5223173 0.8264601 0.6039820 0.5460710 0.2282013 0.7473185
## 2 0.4535404 0.8002493 0.6651733 0.3609305 0.2252915 0.3026661 0.5788398
## 3 0.5229057 0.8532762 0.7557138 0.4049758 0.4928607 0.4109288 0.6887079
## 4 0.4823768 0.8068388 0.8188515 0.8687583 0.4776356 0.7689434 0.7898817
## 5 0.5474553 0.4401289 0.6338240 0.6970505 0.6145062 0.5235850 0.4480877
## 6 0.8061439 0.4710821 0.8731260 0.6773543 0.2657005 0.2898519 0.7116113
##          43        44        45        46        47        48        49
## 1 0.7724649 0.1898354 0.1209358 0.1530435 0.3431442 0.4151619 0.2655463
## 2 0.6235279 0.3100927 0.3006274 0.2286705 0.2604391 0.1895212 0.3825062
## 3 0.7254813 0.5320773 0.3616875 0.1397689 0.2331029 0.1867576 0.4461689
## 4 0.8753426 0.4179388 0.4071622 0.7298923 0.1356029 0.2955204 0.5759310
## 5 0.5996756 0.6755641 0.4693752 0.7606645 0.5795695 0.6302619 0.8158603
## 6 0.9171399 0.1465798 0.2978023 0.4927868 0.2193605 0.2681168 0.2444326
##          50        51          52        53        54        55        56
## 1 0.7542646 0.3571783 0.005958178 0.7555582 0.5987478 0.6860006 0.6279628
## 2 0.5804608 0.5145344 0.773198682 0.6261976 0.6863734 0.3022054 0.5924487
## 3 0.7460427 0.6520767 0.887557241 0.6954348 0.7370605 0.2859430 0.7138417
```

```
## 4 0.6840051 0.3745174 0.202339004 0.8025156 0.6518282 0.8768876 0.7919490
## 5 0.5483860 0.7412892 0.693553719 0.2200268 0.2810707 0.5814088 0.2483926
## 6 0.7471508 0.1958125 0.782984623 0.7311622 0.6750314 0.5440910 0.7375961
##           57         58         59          60         61         62         63
## 1 0.56199985 0.6101566 0.3914218 0.01693461 0.3852755 0.4994856 0.4815546
## 2 0.57910712 0.6436872 0.4334141 0.16218372 0.2769118 0.5407815 0.3618914
## 3 0.67406591 0.6894538 0.4168162 0.17089216 0.2153814 0.7608646 0.3176590
## 4 0.74846416 0.7859733 0.0891590 0.28476904 0.2263688 0.2866464 0.2171926
## 5 0.03922522 0.3200729 0.7333222 0.58738324 0.5606921 0.8417709 0.7849704
## 6 0.64112050 0.6538304 0.2697308 0.11638872 0.3103416 0.6009278 0.3505919
##           64         65         66         67         68         69         70
## 1 0.1137891 0.2200364 0.4418674 0.2250408 0.2299410 0.4838123 0.2309129
## 2 0.6305563 0.5751043 0.4433679 0.6238893 0.6725336 0.4575897 0.5771266
## 3 0.6937444 0.6762452 0.5817876 0.7151988 0.6958088 0.2220223 0.6672334
## 4 0.1505905 0.2704983 0.3849484 0.3393258 0.3047188 0.3113771 0.2555332
## 5 0.6307692 0.7852675 0.5298483 0.7171646 0.6976254 0.8139527 0.6691873
## 6 0.1307633 0.3111088 0.7874459 0.4222490 0.5021210 0.5377189 0.3695351
##           71         72         73         74          75
## 1 0.3352198 0.2666322 0.3517610 0.4063311 0.32591896
## 2 0.4936682 0.4007847 0.5535664 0.7355449 0.41722103
## 3 0.6093995 0.4197364 0.3547702 0.8030353 0.54463451
## 4 0.1617947 0.1789714 0.0629351 0.5099668 0.06620799
## 5 0.6123069 0.2783467 0.8025793 0.8042854 0.54711275
## 6 0.5115109 0.5367194 0.3698399 0.5938658 0.55493889
```

```
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 0.3842069 0.5626552  0.4573103 0.4800000 0.3342759
## LTEE.5..2  0.3842069 0.0000000 0.5486897  0.5174483 0.2984828 0.4882069
## LTEE.5..8  0.5626552 0.5486897 0.0000000  0.2533448 0.6330345 0.5439310
## LTEE2.5.13 0.4573103 0.5174483 0.2533448  0.0000000 0.4668621 0.4562759
## LTEE.5..4  0.4800000 0.2984828 0.6330345  0.4668621 0.0000000 0.4647241
## LTEE.5..5  0.3342759 0.4882069 0.5439310  0.4562759 0.4647241 0.0000000
##             LTEE5.11 LTEE.5..6  LTEE5.12 LTEE.5..3   LTEE5.9 LTEE.6..1
## LTEE.5..1  0.9394828 0.4453793 0.4463448 0.9478966 0.8041724 0.5291379
## LTEE.5..2  0.9063448 0.5590345 0.4936207 0.6040690 0.7741379 0.6258966
## LTEE.5..8  0.3981724 0.6484483 0.2270690 0.8951034 0.2637241 0.6665172
## LTEE2.5.13 0.5267241 0.4766207 0.1287586 0.9116207 0.4034138 0.7359310
## LTEE.5..4  0.9110690 0.4816897 0.4621034 0.5029310 0.8131724 0.7794483
## LTEE.5..5  0.9054483 0.2583448 0.4427241 0.9440690 0.7774483 0.6414483
##            LTEE.6..2 LTEE.6..8 LTEE2.6.13 LTEE.6..4 LTEE.6..5 LTEE.6..6
## LTEE.5..1  0.5459310 0.5052069  0.9915862 0.4441724 0.6021379 0.4248621
## LTEE.5..2  0.1853448 0.5368966  0.9403793 0.2708621 0.4222069 0.5523103
## LTEE.5..8  0.5650690 0.1604138  0.4919310 0.5213448 0.5729655 0.5956552
## LTEE2.5.13 0.5319310 0.1396897  0.5738276 0.4789310 0.6697241 0.4587931
## LTEE.5..4  0.2201034 0.4966207  0.9485172 0.1965517 0.5293793 0.4569655
## LTEE.5..5  0.5507931 0.4803103  0.9903448 0.3924828 0.5294138 0.2096207
##             LTEE6.12 LTEE.6..3   LTEE6.9   LTEE7.2 LTEE.7..1   LTEE7.4
## LTEE.5..1  0.7870690 0.8893103 0.7473448 0.4812759 0.1373103 0.5497931
## LTEE.5..2  0.7518276 0.5472069 0.6969655 0.5048966 0.3849310 0.3006552
## LTEE.5..8  0.2510000 0.8287931 0.2251034 0.6595517 0.5712414 0.6506207
## LTEE2.5.13 0.3704483 0.8450345 0.3456897 0.4778276 0.4937586 0.5243793
## LTEE.5..4  0.7761724 0.4558276 0.7588966 0.4224828 0.5380345 0.1053793
## LTEE.5..5  0.7552759 0.8806897 0.7458966 0.2903448 0.4125172 0.5406897
##              LTEE7.5 LTEE.7..6   LTEE7.3 LTEE.8..1 LTEE.8..2 LTEE.8..8
## LTEE.5..1  0.4513103 0.4240690 0.4492414 0.2180690 0.4826207 0.4958276
## LTEE.5..2  0.4096897 0.5495172 0.4466207 0.3875862 0.5449310 0.5738621
## LTEE.5..8  0.6276897 0.5932759 0.6325862 0.5713793 0.6637241 0.3295862
## LTEE2.5.13 0.4530345 0.4627586 0.4570000 0.4854138 0.4883448 0.2292414
## LTEE.5..4  0.2944828 0.4440345 0.3861379 0.5298966 0.4538621 0.4752759
## LTEE.5..5  0.2622759 0.1852069 0.2736552 0.3918966 0.2885517 0.3886897
##            LTEE2.8.13 LTEE.8..4 LTEE.8..5  LTEE8.11 LTEE.8..6  LTEE8.12
## LTEE.5..1   0.5013103 0.4967586 0.4610000 0.5842414 0.4597931 0.5039310
## LTEE.5..2   0.5803103 0.6091034 0.4511379 0.5824138 0.5501724 0.6033793
## LTEE.5..8   0.4272759 0.6992069 0.6331034 0.1409655 0.6150690 0.7010345
## LTEE2.5.13  0.2392759 0.5243103 0.4604483 0.3111034 0.4627241 0.5151379
## LTEE.5..4   0.4854828 0.5008966 0.3526552 0.7037241 0.4381034 0.5037241
## LTEE.5..5   0.3056207 0.3002759 0.2649310 0.5614138 0.2412414 0.3084483
##            LTEE.8..3   LTEE8.9 LTEE.9..1 LTEE.9..2 LTEE.9..8 LTEE2.9.13
## LTEE.5..1  0.8728621 0.9795517 0.3121379 0.4952414 0.4654138  0.5063448
## LTEE.5..2  0.5274138 0.9257931 0.4121724 0.5156897 0.5519310  0.5975517
## LTEE.5..8  0.8242759 0.4681379 0.5781379 0.6710000 0.2624138  0.5212069
## LTEE2.5.13 0.8395862 0.5662069 0.4557586 0.4915862 0.1670345  0.3312414
## LTEE.5..4  0.4334483 0.9349655 0.4667931 0.4260345 0.4442069  0.4997931
## LTEE.5..5  0.8653448 0.9623448 0.1919655 0.2999655 0.4171034  0.3115517
##            LTEE.9..4 LTEE.9..5  LTEE9.11 LTEE2.9.12 LTEE.9..3   LTEE9.9
## LTEE.5..1  0.4094828 0.7788621 0.7630690  0.5015172 0.5886897 0.6262069
## LTEE.5..2  0.5275172 0.4914483 0.7054138  0.6097586 0.2402069 0.5826207
## LTEE.5..8  0.6146207 0.7333448 0.2645862  0.7039310 0.5993793 0.1090000
```

```
## LTEE2.5.13 0.4625862 0.7960000 0.4588621  0.5153103 0.5628966 0.2484828
## LTEE.5..4  0.4694138 0.3937241 0.8214828  0.5082759 0.2072759 0.6501724
## LTEE.5..5  0.2184828 0.7494828 0.7626897  0.3066207 0.5917586 0.6272069
##            LTEE.13..1 LTEE.13..2 LTEE.13..8 LTEE.13..4 LTEE.13..5 LTEE13.11
## LTEE.5..1   0.1095172  0.7180000  0.3622414  0.7884828  0.8171379 0.9128276
## LTEE.5..2   0.3869655  0.3509310  0.4416552  0.8251379  0.5165862 0.8743103
## LTEE.5..8   0.5697586  0.6724828  0.2303793  0.7734828  0.7825172 0.3746207
## LTEE2.5.13  0.4795862  0.7416552  0.1422069  0.8205517  0.8057586 0.5011379
## LTEE.5..4   0.5243793  0.4044483  0.4505517  0.8434483  0.4024828 0.8914483
## LTEE.5..5   0.3903448  0.7194828  0.3768966  0.6920690  0.8131034 0.8855517
##            LTEE.13..6 LTEE.13..3  LTEE13.9 LTEE.16..1 LTEE.16..2 LTEE.16..8
## LTEE.5..1   0.4336207  0.9742414 0.6365517  0.2853103  0.9474828  0.6190690
## LTEE.5..2   0.3464138  0.6391724 0.6017586  0.4932414  0.5884828  0.5785862
## LTEE.5..8   0.5461724  0.9266552 0.1786897  0.5698276  0.9050345  0.1756207
## LTEE2.5.13  0.6375517  0.9445862 0.3355517  0.4576552  0.9149310  0.2036207
## LTEE.5..4   0.5513103  0.5376207 0.7500690  0.4800690  0.5774138  0.5846552
## LTEE.5..5   0.5624138  0.9734138 0.6834828  0.3449310  0.9459655  0.6181379
##            LTEE.16..4 LTEE.16..5 LTEE16.11 LTEE.16..6 LTEE2.16.12 LTEE.16..3
## LTEE.5..1   0.4896897  0.4828621 0.9383448  0.4628966   0.4948276  0.4837931
## LTEE.5..2   0.6116552  0.3880000 0.8973793  0.4169655   0.5827241  0.3546897
## LTEE.5..8   0.6806552  0.6447241 0.4133448  0.6263793   0.5694828  0.6450000
## LTEE2.5.13  0.5121034  0.4805172 0.5105517  0.4552414   0.3782069  0.4778621
## LTEE.5..4   0.4892414  0.2000345 0.8941379  0.3282414   0.4936897  0.2793103
## LTEE.5..5   0.2814483  0.3188621 0.9209655  0.2644138   0.3032069  0.2941724
##             LTEE16.9  LTEE17.8 LTEE2.17.13  LTEE17.5 LTEE2.17.11  LTEE17.6
## LTEE.5..1  0.4083103 0.6605862   0.5024483 0.5807931   0.7798276 0.4907586
## LTEE.5..2  0.4965172 0.6274828   0.5920690 0.3232069   0.7584138 0.4756207
## LTEE.5..8  0.4667241 0.1345517   0.5181379 0.5477931   0.3811379 0.6583448
## LTEE2.5.13 0.3156207 0.2355517   0.3293103 0.6466552   0.4532069 0.4803448
## LTEE.5..4  0.4703793 0.6287586   0.4929310 0.2550000   0.8722759 0.3843103
## LTEE.5..5  0.2338966 0.6513103   0.3075517 0.5354483   0.7749310 0.2935172
##            LTEE2.17.12  LTEE17.3 LTEE2.17.9
## LTEE.5..1    0.4992759 0.8141724  0.4488966
## LTEE.5..2    0.5794138 0.4703793  0.5416552
## LTEE.5..8    0.4046207 0.7668966  0.4643103
## LTEE2.5.13   0.2130000 0.7803793  0.2785517
## LTEE.5..4    0.4807931 0.3612759  0.4664483
## LTEE.5..5    0.3204828 0.8123448  0.2647931
```

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="bray",na.rm=TRUE)
  microbiomedist = vegdist(microbiome, method="bray",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)
```

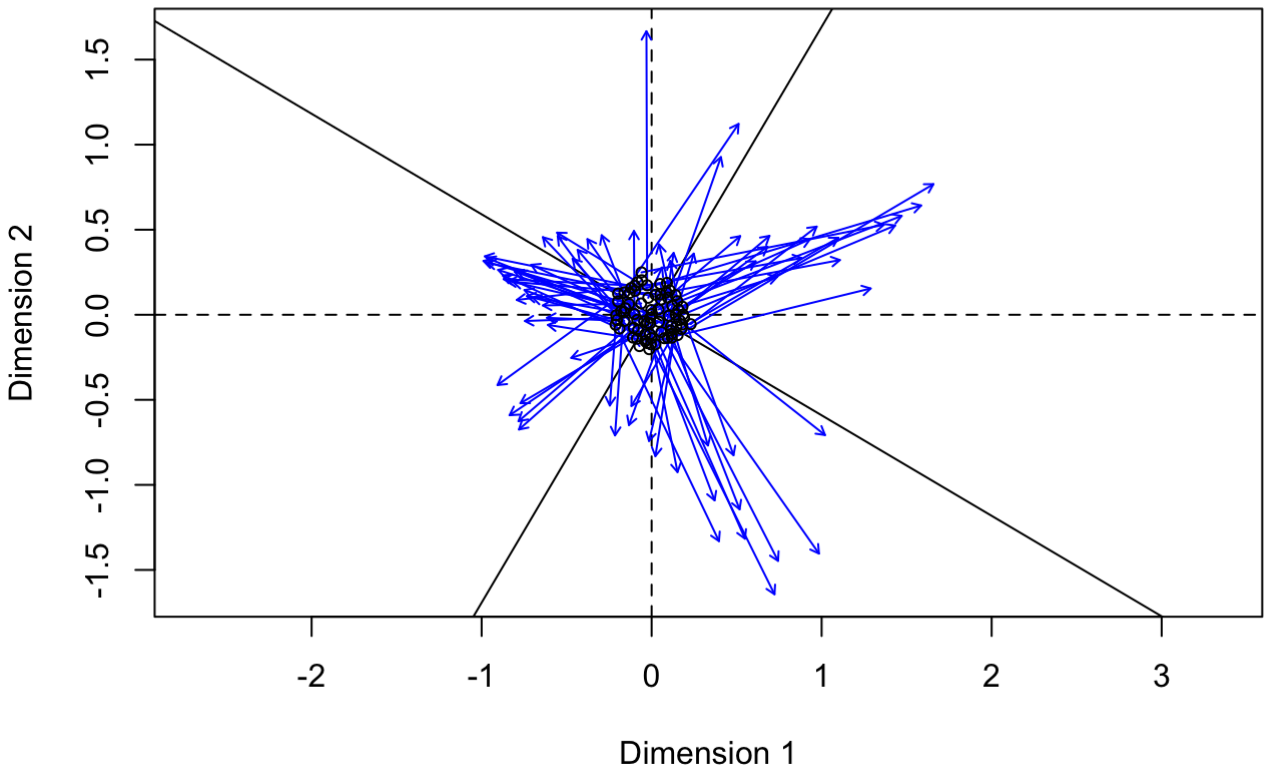
```
## Monte-Carlo test
## Call: mantelnoneuclid(m1 = m1, m2 = m2, nrepet = nrepet)
##
## Observation: 0.05027329
##
## Based on 999 replicates
## Simulated p-value: 0.051
## Alternative hypothesis: greater
##
##       Std.Obs   Expectation      Variance
##  1.6476086169 -0.0010031393  0.0009685621
```

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:

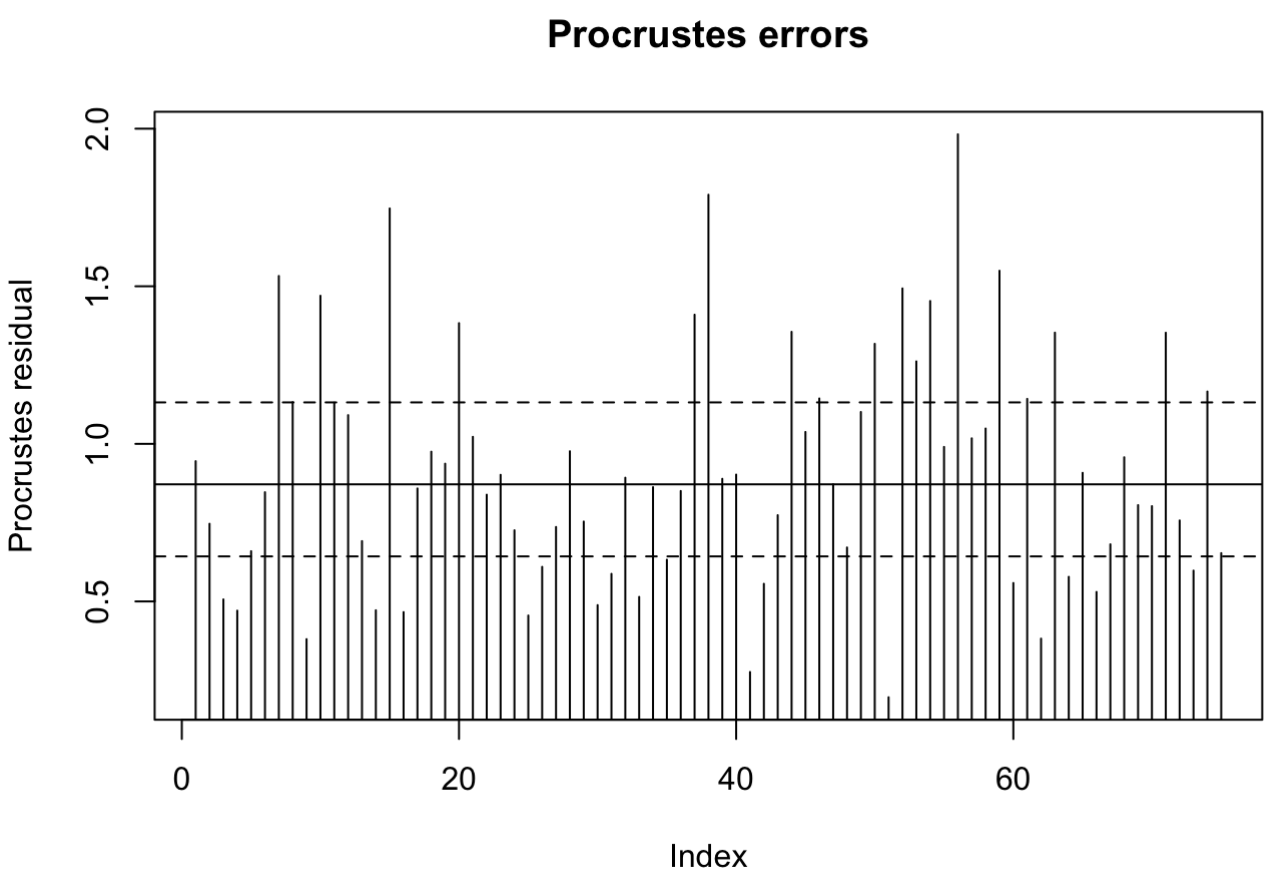
```
# Do a MDS on the distance matrices
microbiomeMDS <- monoMDS(microbiomedist)
metaboliteMDS <- monoMDS(metabolitedist)
# Perform Procrustes
vare.proc <- procrustes(microbiomeMDS, metaboliteMDS)
summary(vare.proc)
```

```
##
## Call:
## procrustes(X = microbiomeMDS, Y = metaboliteMDS)
##
## Number of objects: 75    Number of dimensions: 2
##
## Procrustes sum of squares:
##   73.0157
## Procrustes root mean squared error:
##  0.9866827
## Quantiles of Procrustes errors:
##       Min        1Q    Median        3Q       Max
## 0.1960251 0.6429023 0.8715726 1.1315531 1.9822264
##
## Rotation matrix:
##            [,1]       [,2]
## [1,] -0.8609270  0.5087284
## [2,] -0.5087284 -0.8609270
##
## Translation of averages:
##              [,1]         [,2]
## [1,] 3.470912e-18 1.463823e-17
##
## Scaling of target:
## [1] 0.1626571
```

```
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
##   0.9450662   0.7464468   0.5064303   0.4708006   0.6594321   0.8465655
##    LTEE5.11   LTEE.5..6    LTEE5.12   LTEE.5..3     LTEE5.9   LTEE.6..1
##   1.5327050   1.1323815   0.3804429   1.4699323   1.1307246   1.0911925
##   LTEE.6..2   LTEE.6..8  LTEE2.6.13   LTEE.6..4   LTEE.6..5   LTEE.6..6
##   0.6914136   0.4720412   1.7469665   0.4661999   0.8584983   0.9751704
##    LTEE6.12   LTEE.6..3     LTEE6.9     LTEE7.2   LTEE.7..1     LTEE7.4
##   0.9370739   1.3830762   1.0223524   0.8387107   0.9017448   0.7257997
##     LTEE7.5   LTEE.7..6     LTEE7.3   LTEE.8..1   LTEE.8..2   LTEE.8..8
##   0.4553721   0.6097626   0.7362923   0.9765499   0.7535367   0.4883638
##  LTEE2.8.13   LTEE.8..4   LTEE.8..5    LTEE8.11   LTEE.8..6    LTEE8.12
##   0.5875572   0.8925737   0.5144841   0.8626785   0.6324716   0.8504956
##   LTEE.8..3     LTEE8.9   LTEE.9..1   LTEE.9..2   LTEE.9..8  LTEE2.9.13
##   1.4098331   1.7904428   0.8891062   0.9024950   0.2761424   0.5559595
##   LTEE.9..4   LTEE.9..5    LTEE9.11  LTEE2.9.12   LTEE.9..3     LTEE9.9
##   0.7737277   1.3554738   1.0377903   1.1441060   0.8715726   0.6712406
##  LTEE.13..1  LTEE.13..2  LTEE.13..8  LTEE.13..4  LTEE.13..5   LTEE13.11
##   1.1012053   1.3174136   0.1960251   1.4932237   1.2617505   1.4532800
##  LTEE.13..6  LTEE.13..3    LTEE13.9  LTEE.16..1  LTEE.16..2  LTEE.16..8
##   0.9903237   1.9822264   1.0175748   1.0486996   1.5493314   0.5585060
##  LTEE.16..4  LTEE.16..5   LTEE16.11  LTEE.16..6 LTEE2.16.12  LTEE.16..3
##   1.1427713   0.3820324   1.3529289   0.5781302   0.9080495   0.5301619
##    LTEE16.9    LTEE17.8 LTEE2.17.13    LTEE17.5 LTEE2.17.11    LTEE17.6
##   0.6812819   0.9574297   0.8056462   0.8024069   1.3526786   0.7569907
## LTEE2.17.12    LTEE17.3  LTEE2.17.9
##   0.5978792   1.1660648   0.6533329
```

```
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):        0.9735
## Correlation in a symmetric Procrustes rotation: 0.1627
## Significance:  0.254
##
## Permutation: free
## Number of permutations: 999
```

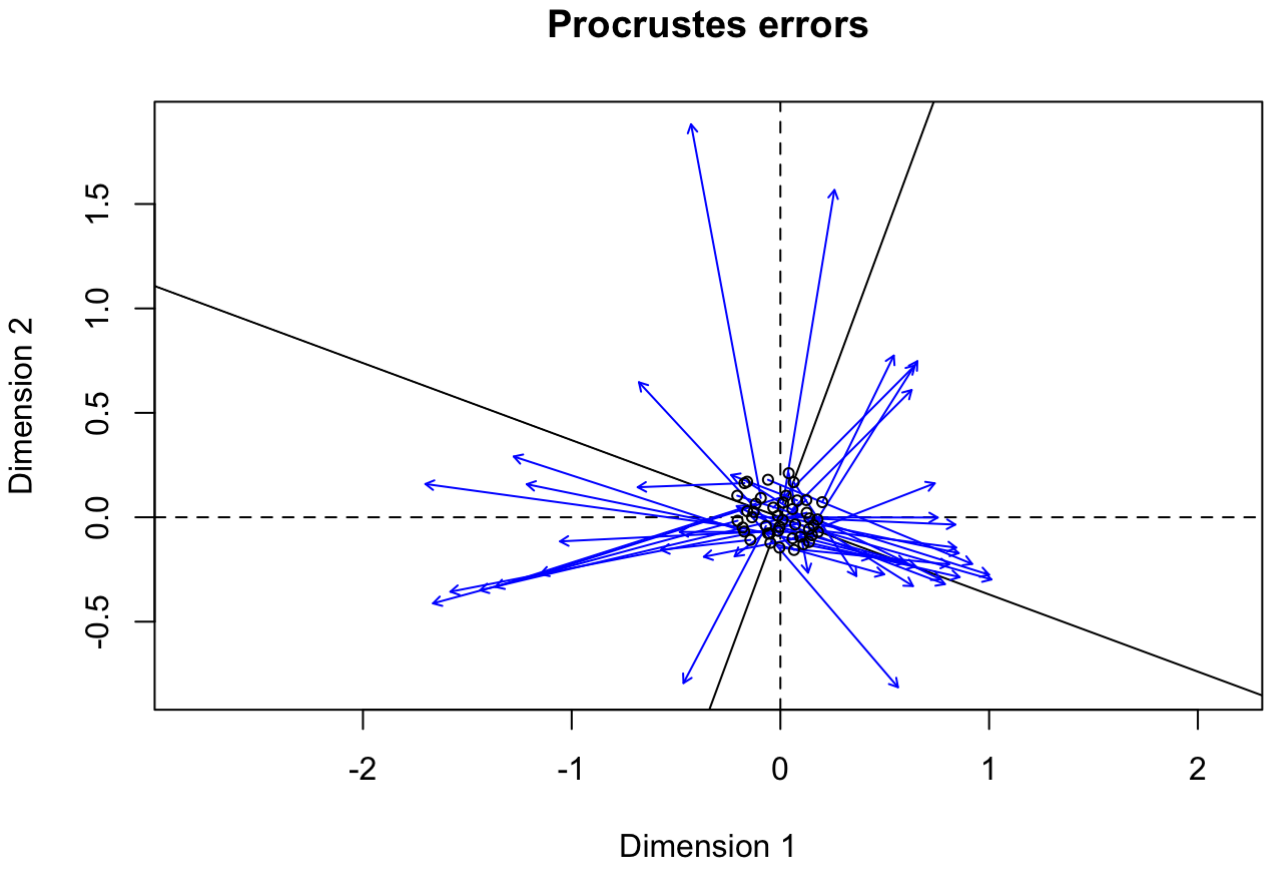
So we can see that the test of significance on the Procrustes rotation is not significant. Let's do the same thing, but write a function to print everything out first:

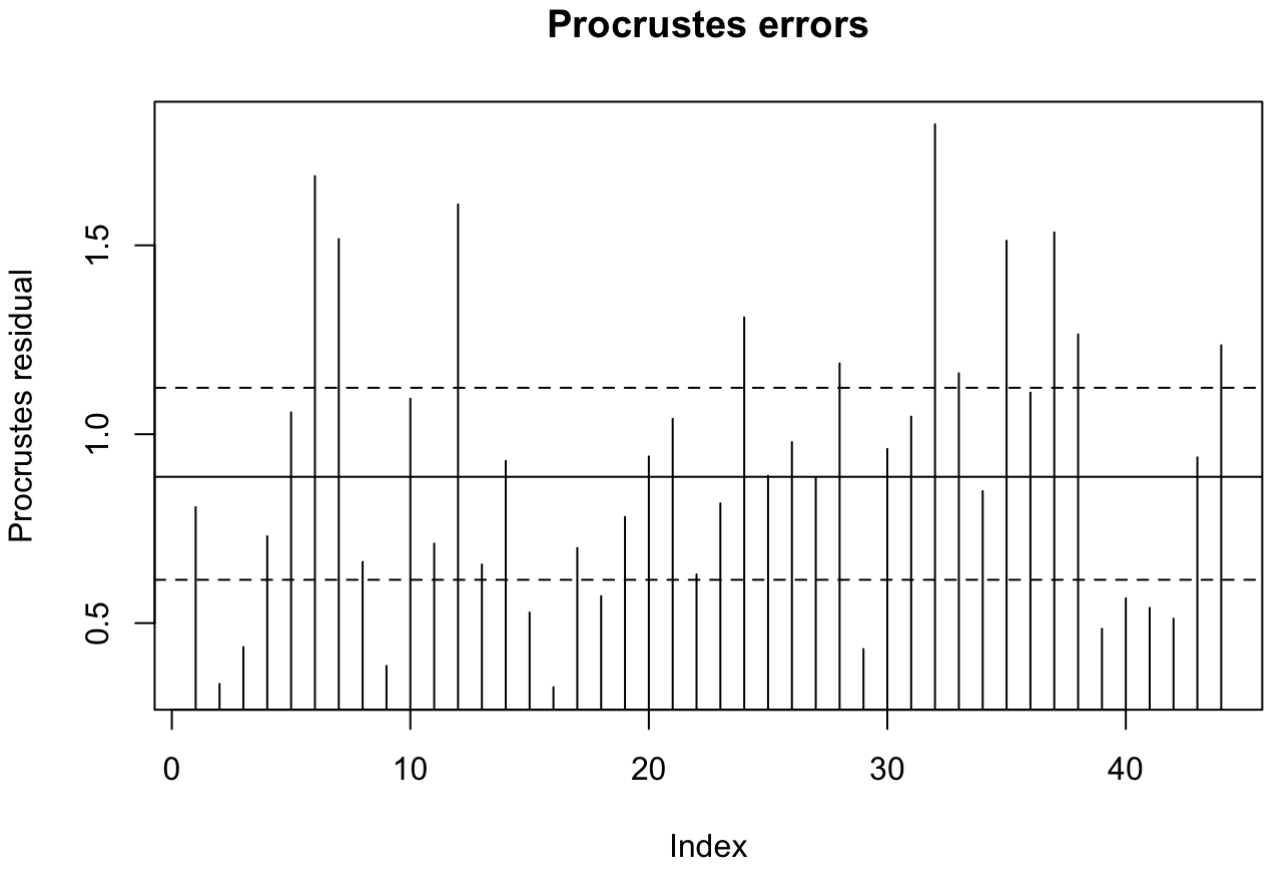
```
procrustesfunct <- function(metabolitedist, microbiomedist) {
  print(mantel.rtest(metabolitedist, microbiomedist, nrepet = 999))
  # Do a MDS on the distance matrices
  microbiomeMDS <- monoMDS(microbiomedist)
  metaboliteMDS <- monoMDS(metabolitedist)
  # Perform Procrustes
  vare.proc <- procrustes(microbiomeMDS, 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:

```
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: -0.0134028
##
## Based on 999 replicates
## Simulated p-value: 0.606
## Alternative hypothesis: greater
##
##      Std.Obs  Expectation     Variance
## -0.303001268  0.001386755  0.002382438
```


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

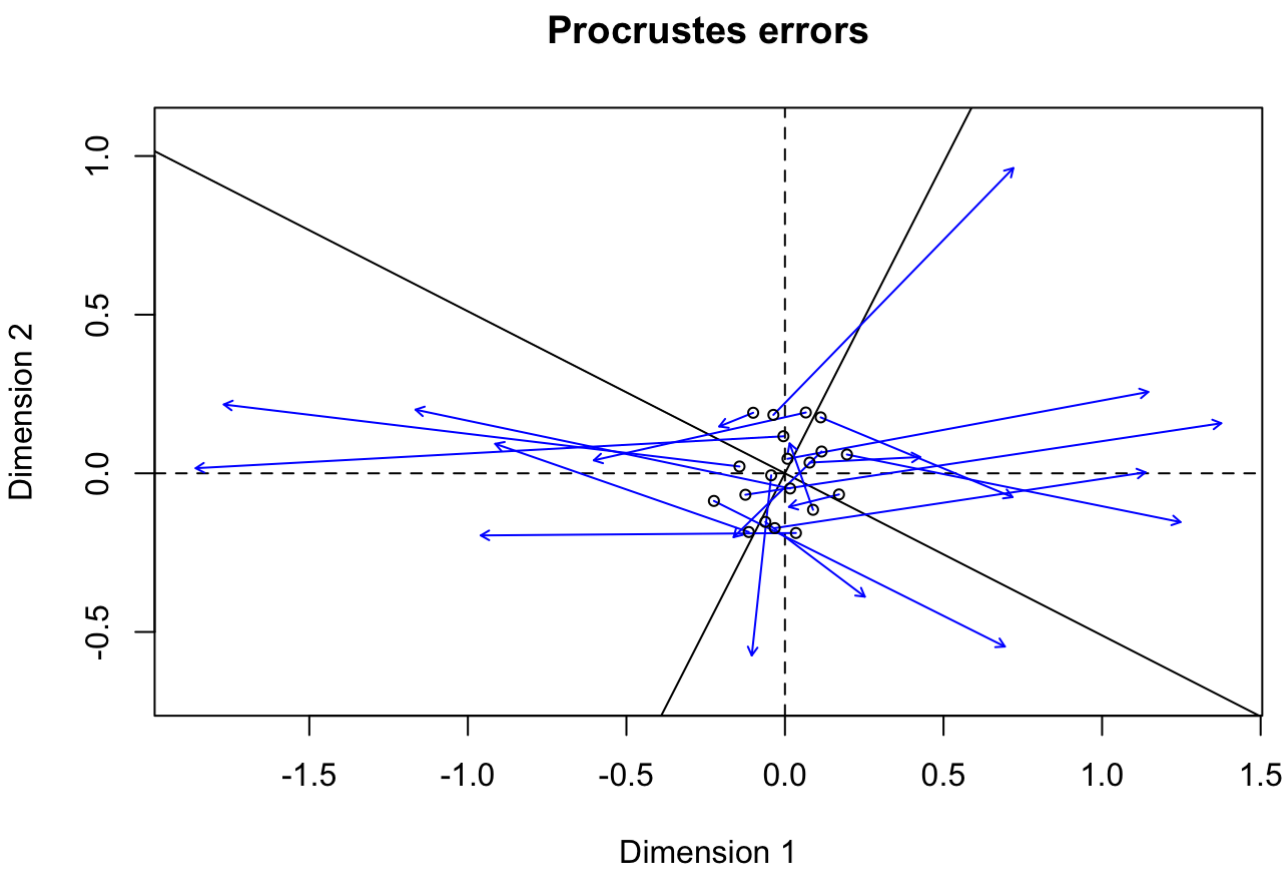

Procrustes errors
Procrustes residual
1.5
1.0
0.5
0
10
20
30
40
Index

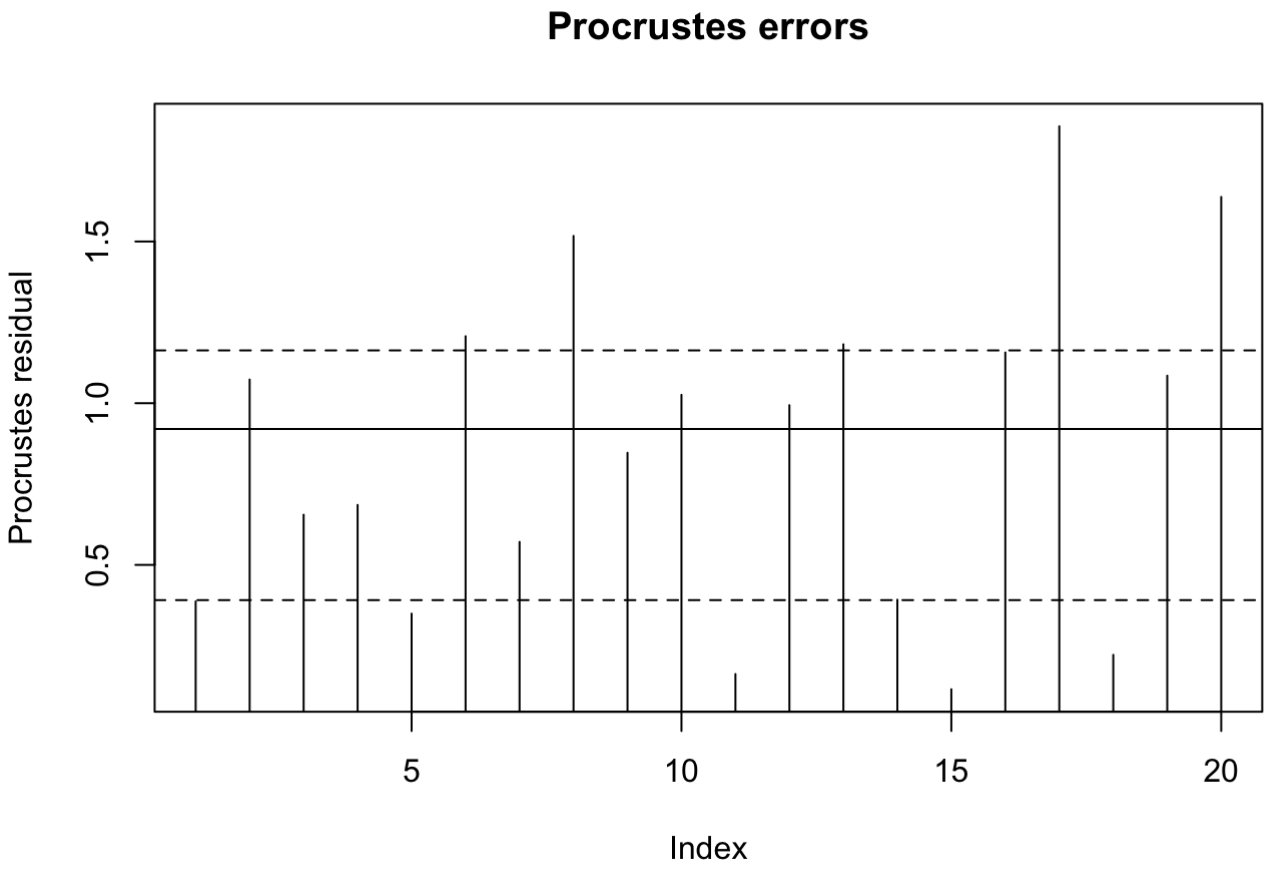
```
##
## Call:
## protest(X = microbiomeMDS, Y = metaboliteMDS, scores = "sites",      permutations = h
ow(nperm = 999))
##
## Procrustes Sum of Squares (m12 squared):        0.9771
## Correlation in a symmetric Procrustes rotation: 0.1513
## Significance:  0.574
##
## Permutation: free
## Number of 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: -0.04793124
##
## Based on 999 replicates
## Simulated p-value: 0.718
## Alternative hypothesis: greater
##
##       Std.Obs   Expectation      Variance
## -0.5805510186  0.0004129967  0.0069343956
```


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

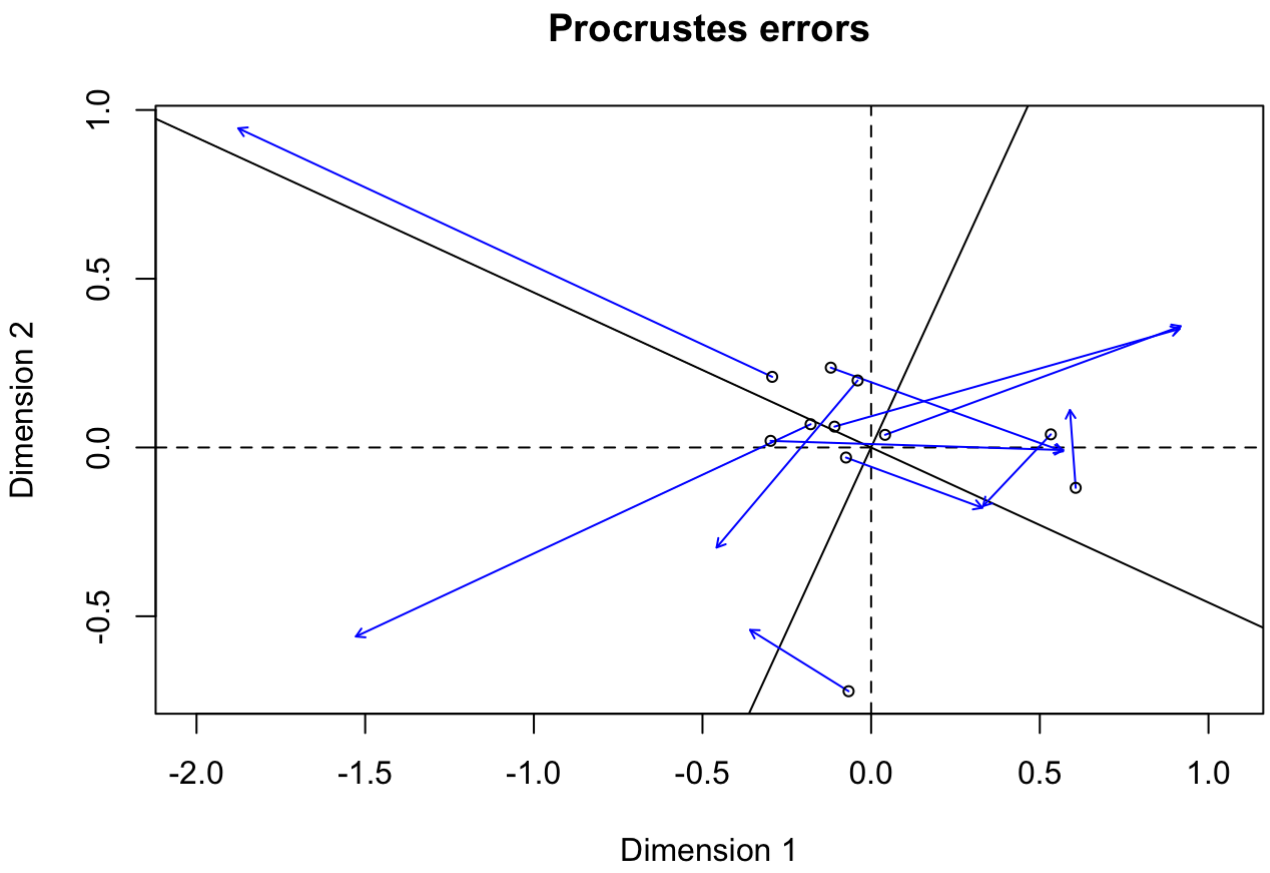

Procrustes errors
Procrustes residual
0.5
1.0
1.5
5
10
15
20
Index

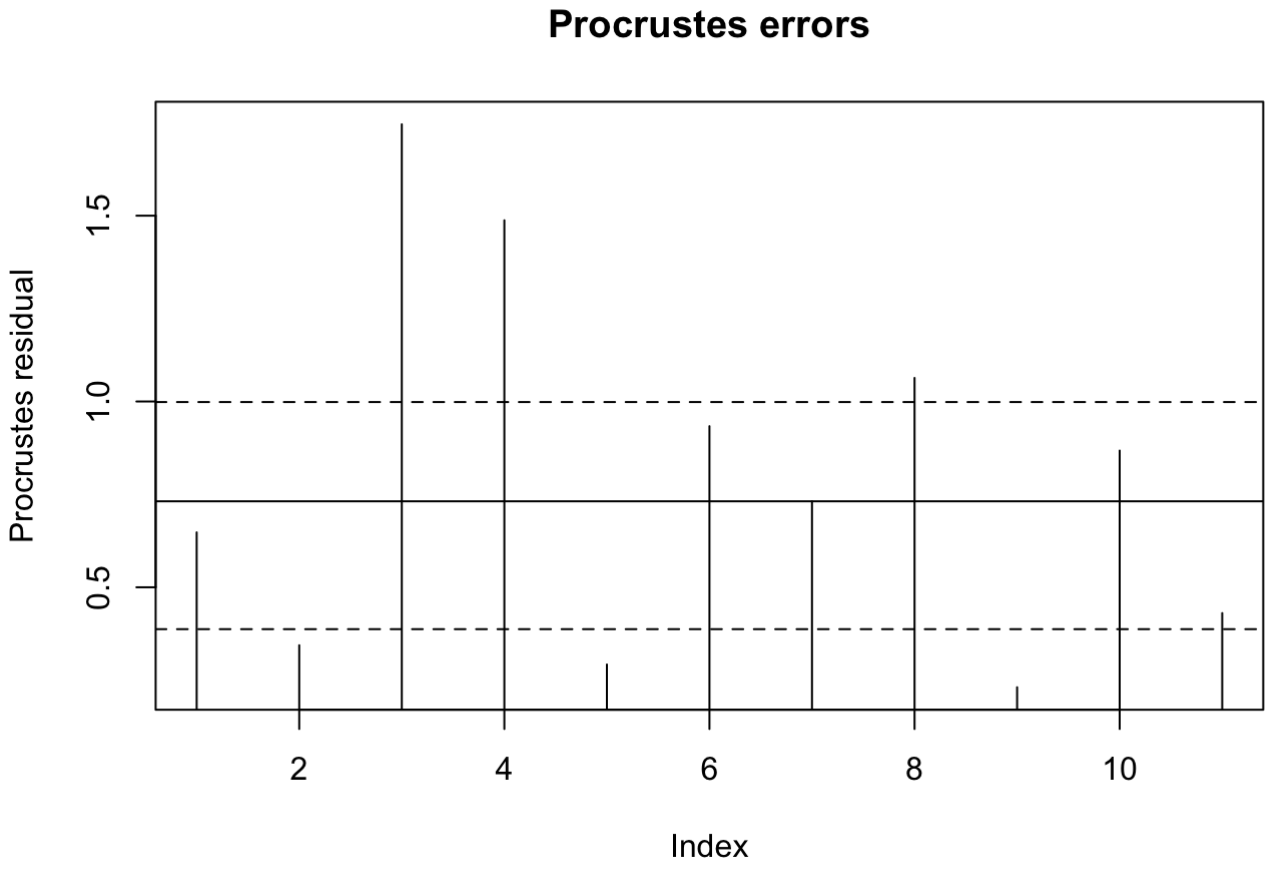
```
##
## Call:
## protest(X = microbiomeMDS, Y = metaboliteMDS, scores = "sites",      permutations = h
ow(nperm = 999))
##
## Procrustes Sum of Squares (m12 squared):        0.9725
## Correlation in a symmetric Procrustes rotation: 0.1657
## Significance:  0.757
##
## Permutation: free
## Number of 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: -0.144668
##
## Based on 999 replicates
## Simulated p-value: 0.671
## Alternative hypothesis: greater
##
##     Std.Obs Expectation    Variance
## -0.58742101 -0.01491157  0.04879316
```


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

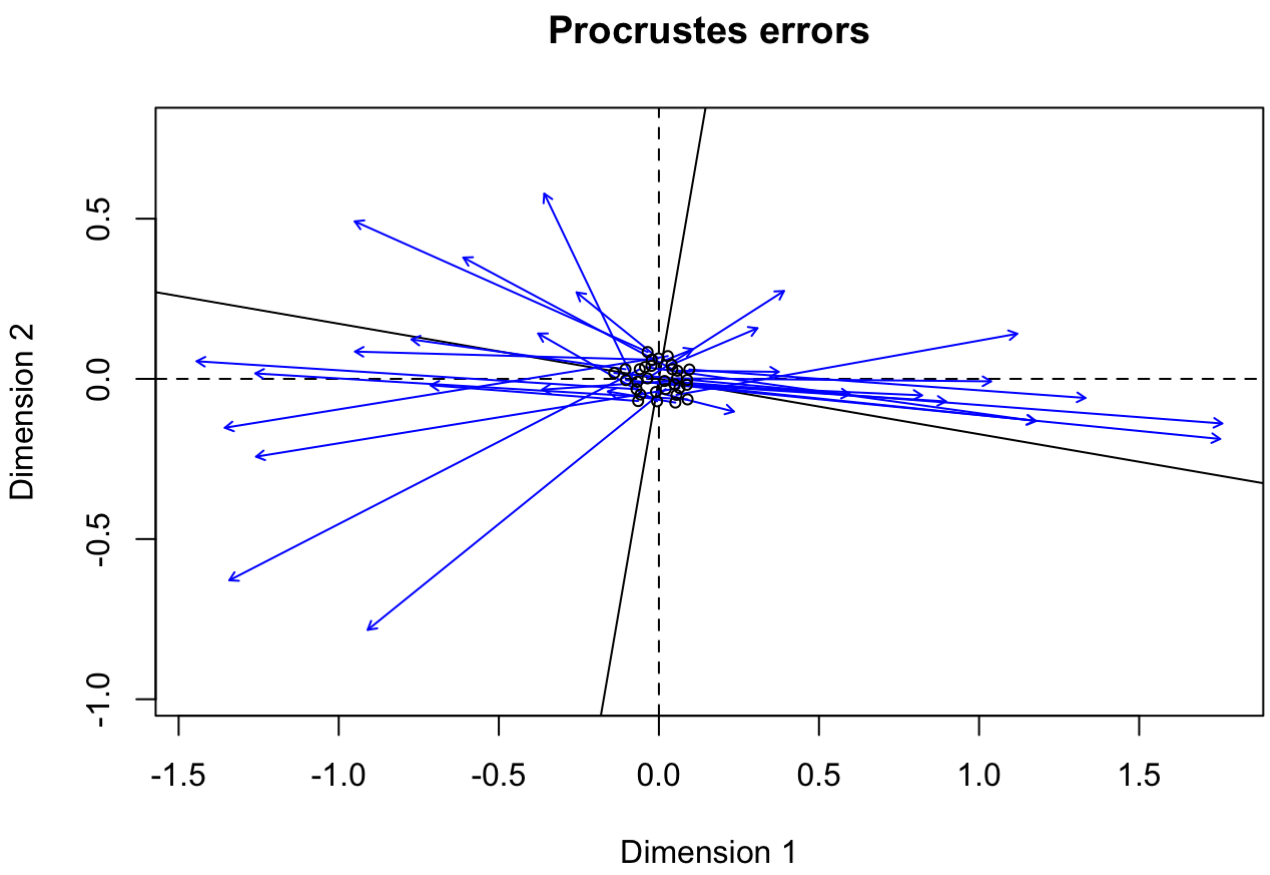

Procrustes errors
Procrustes residual
1.5
1.0
0.5
2
4
6
8
10
Index

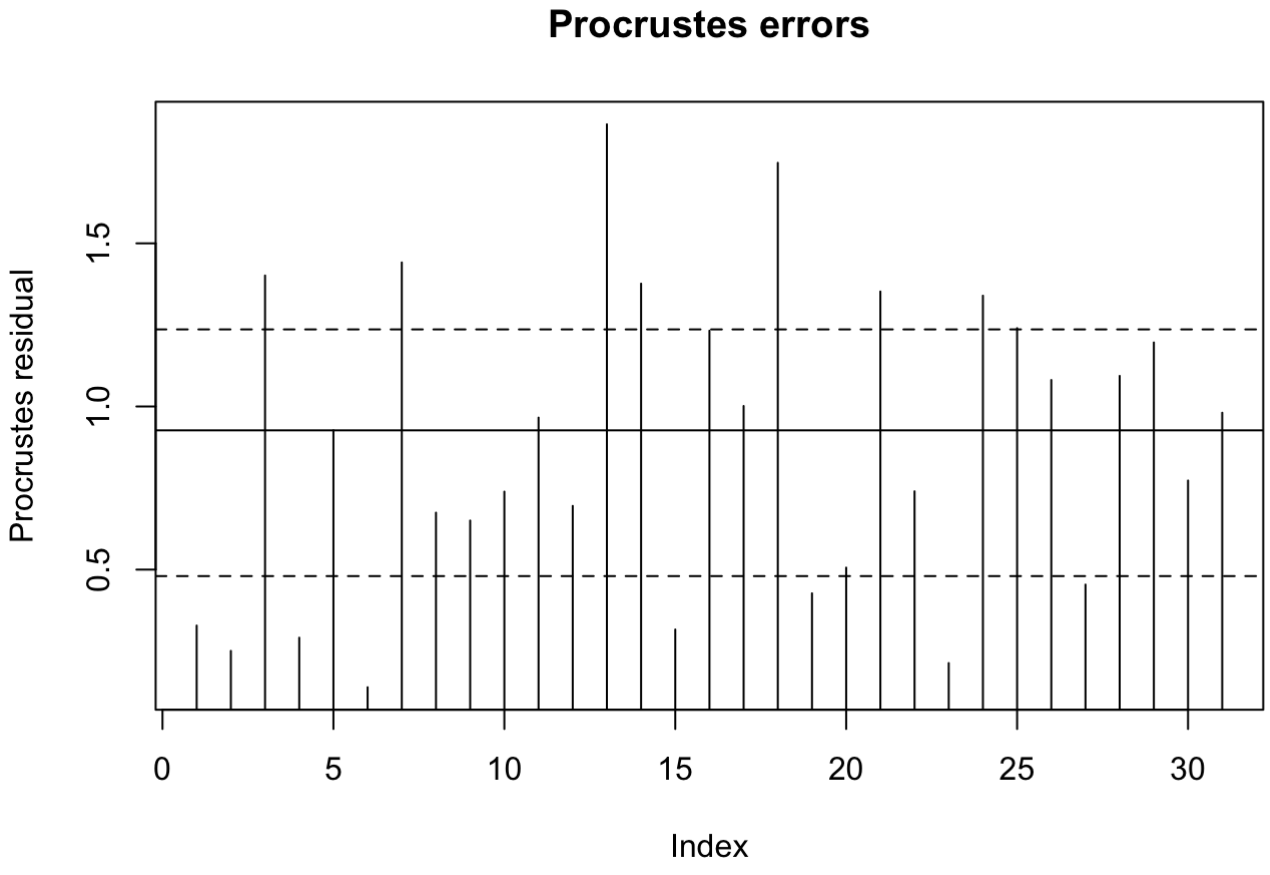
```
##
## Call:
## protest(X = microbiomeMDS, Y = metaboliteMDS, scores = "sites",      permutations = h
ow(nperm = 999))
##
## Procrustes Sum of Squares (m12 squared):        0.8559
## Correlation in a symmetric Procrustes rotation: 0.3797
## Significance:  0.39
##
## Permutation: free
## Number of 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="bray",na.rm=TRUE)
microbiomedist = vegdist(microbiome, method="bray",na.rm=TRUE)
procrustesfunct(metabolitedist, microbiomedist)
```

```
## Monte-Carlo test
## Call: mantelnoneuclid(m1 = m1, m2 = m2, nrepet = nrepet)
##
## Observation: -0.05774766
##
## Based on 999 replicates
## Simulated p-value: 0.859
## Alternative hypothesis: greater
##
##       Std.Obs   Expectation      Variance
## -1.1016672210 -0.0009969932  0.0026536341
```


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


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

```
##
## Call:
## protest(X = microbiomeMDS, Y = metaboliteMDS, scores = "sites",      permutations = h
ow(nperm = 999))
##
## Procrustes Sum of Squares (m12 squared):        0.9941
## Correlation in a symmetric Procrustes rotation: 0.07661
## Significance:  0.933
##
## Permutation: free
## Number of permutations: 999
```