# Spectra-trait PLSR example using leaf-level spectra and leaf nitrogen content (Narea, g/m2) data from 36 species growing in Rosa rugosa invaded coastal grassland communities in Belgium. Bootstrap validation example

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### Overview

This is an R Markdown Notebook to illustrate how to retrieve a dataset from the EcoSIS spectral database, choose the "optimal" number of plsr components, and fit a plsr model for leaf nitrogen content (Narea, g/m2)

### Getting Started

#### Load libraries

```
list.of.packages <- c("pls","dplyr","here","plotrix","ggplot2","gridExtra","spectratrait")
invisible(lapply(list.of.packages, library, character.only = TRUE))
```

```
## Warning: package 'pls' was built under R version 4.3.1
##
## Attaching package: 'pls'
## The following object is masked from 'package:stats':
##
##     loadings
## Warning: package 'dplyr' was built under R version 4.3.1
##
## Attaching package: 'dplyr'
## The following objects are masked from 'package:stats':
##
##     filter, lag
## The following objects are masked from 'package:base':
##
##     intersect, setdiff, setequal, union
## here() starts at /Users/sserbin/Library/CloudStorage/OneDrive-NASA/Data/Github/spectratrait
## Warning: package 'plotrix' was built under R version 4.3.1
## Warning: package 'ggplot2' was built under R version 4.3.1
##
## Attaching package: 'gridExtra'
```

```
## The following object is masked from 'package:dplyr':
##
##     combine
```

### Setup other functions and options

```
### Setup options

# Script options
pls::pls.options(plsralg = "oscorespls")
pls::pls.options("plsralg")
```

```
## $plsralg
## [1] "oscorespls"
```

```
# Default par options
opar <- par(no.readonly = T)

# What is the target variable?
inVar <- "Narea_g_m2"

# What is the source dataset from EcoSIS?
ecosis_id <- "9db4c5a2-7eac-4e1e-8859-009233648e89"

# Specify output directory, output_dir
# Options:
# tempdir - use a OS-specified temporary directory
# user defined PATH - e.g. "~/scratch/PLSR"
output_dir <- "tempdir"
```

### Set working directory (scratch space)

```
## [1] "/private/var/folders/th/fpt_z3417gn8xgply92pvy6r0000gq/T/Rtmp41ruG8"
```

### Grab data from EcoSIS

```
print(paste0("Output directory: ",getwd()))  # check wd
```

```
## [1] "Output directory: /Users/sserbin/Library/CloudStorage/OneDrive-NASA/Data/Github/spectratrait/vig
```

```
dat_raw <- spectratrait::get_ecosis_data(ecosis_id = ecosis_id)
```

```
## [1] "**** Downloading Ecosis data ****"
```

```
## Downloading data...
```

```
## Rows: 256 Columns: 2164
## -- Column specification --------------------------------------------------------
## Delimiter: ","
## chr    (4): Latin Species, ids, plot code, species code
## dbl (2160): Cw/EWT (cm3/cm2), Leaf area (mm2), Leaf calcium content per leaf...
##
## i Use `spec()` to retrieve the full column specification for this data.
## i Specify the column types or set `show_col_types = FALSE` to quiet this message.
## Download complete!
```

```r
head(dat_raw)
```

```
## # A tibble: 6 x 2,164
##   `Cw/EWT (cm3/cm2)` `Latin Species`    `Leaf area (mm2)` Leaf calcium content~1
##                <dbl> <chr>                          <dbl>                  <dbl>
## 1            0.00887 Arrhenatherum ela~              696.                 0.0291
## 2            0.00824 Bromus sterilis                 447.                 0.0230
## 3            0.0280  Jacobaea vulgaris              2418.                 0.0950
## 4            0.0106  Rubus caesius                  5719.                 0.0700
## 5            0.00851 Arrhenatherum ela~              671.                 0.0286
## 6            0.0153  Crepis capillaris              1401.                 0.0470
## # i abbreviated name: 1: `Leaf calcium content per leaf area (mg/mm2)`
## # i 2,160 more variables:
## #   `Leaf magnesium content per leaf area (mg/mm2)` <dbl>,
## #   `Leaf mass per area (g/cm2)` <dbl>,
## #   `Leaf nitrogen content per leaf area (mg/mm2)` <dbl>,
## #   `Leaf phosphorus content per leaf area (mg/mm2)` <dbl>,
## #   `Leaf potassium content per leaf area (mg/mm2)` <dbl>, ...
```

```r
names(dat_raw)[1:40]
```

```
##  [1] "Cw/EWT (cm3/cm2)"
##  [2] "Latin Species"
##  [3] "Leaf area (mm2)"
##  [4] "Leaf calcium content per leaf area (mg/mm2)"
##  [5] "Leaf magnesium content per leaf area (mg/mm2)"
##  [6] "Leaf mass per area (g/cm2)"
##  [7] "Leaf nitrogen content per leaf area (mg/mm2)"
##  [8] "Leaf phosphorus content per leaf area (mg/mm2)"
##  [9] "Leaf potassium content per leaf area (mg/mm2)"
## [10] "Plant height vegetative (cm)"
## [11] "ids"
## [12] "plot code"
## [13] "species code"
## [14] "350"
## [15] "351"
## [16] "352"
## [17] "353"
## [18] "354"
## [19] "355"
## [20] "356"
## [21] "357"
## [22] "358"
## [23] "359"
## [24] "360"
## [25] "361"
## [26] "362"
## [27] "363"
## [28] "364"
## [29] "365"
## [30] "366"
## [31] "367"
## [32] "368"
## [33] "369"
```

```
## [34] "370"
## [35] "371"
## [36] "372"
## [37] "373"
## [38] "374"
## [39] "375"
## [40] "376"
```

### Create full plsr dataset

```
### Create plsr dataset
Start.wave <- 500
End.wave <- 2400
wv <- seq(Start.wave,End.wave,1)
Spectra <- as.matrix(dat_raw[,names(dat_raw) %in% wv])
colnames(Spectra) <- c(paste0("Wave_",wv))
sample_info <- dat_raw[,names(dat_raw) %notin% seq(350,2500,1)]
head(sample_info)
```

```
## # A tibble: 6 x 13
##   `Cw/EWT (cm3/cm2)` `Latin Species`    `Leaf area (mm2)` Leaf calcium content~1
##                <dbl> <chr>                          <dbl>                  <dbl>
## 1            0.00887 Arrhenatherum ela~              696.                 0.0291
## 2            0.00824 Bromus sterilis                 447.                 0.0230
## 3            0.0280  Jacobaea vulgaris              2418.                 0.0950
## 4            0.0106  Rubus caesius                  5719.                 0.0700
## 5            0.00851 Arrhenatherum ela~              671.                 0.0286
## 6            0.0153  Crepis capillaris              1401.                 0.0470
## # i abbreviated name: 1: `Leaf calcium content per leaf area (mg/mm2)`
## # i 9 more variables: `Leaf magnesium content per leaf area (mg/mm2)` <dbl>,
## #   `Leaf mass per area (g/cm2)` <dbl>,
## #   `Leaf nitrogen content per leaf area (mg/mm2)` <dbl>,
## #   `Leaf phosphorus content per leaf area (mg/mm2)` <dbl>,
## #   `Leaf potassium content per leaf area (mg/mm2)` <dbl>,
## #   `Plant height vegetative (cm)` <dbl>, ids <chr>, `plot code` <chr>, ...
```

```
sample_info2 <- sample_info %>%
  select(Plant_Species=`Latin Species`,Species_Code=`species code`,Plot=`plot code`,
         Narea_mg_mm2=`Leaf nitrogen content per leaf area (mg/mm2)`)
sample_info2 <- sample_info2 %>%
#  mutate(Narea_g_m2=Narea_mg_mm2*(0.001/1e-6)) # based on orig units should be this but conversion wro
  mutate(Narea_g_m2=Narea_mg_mm2*100) # this assumes orig units were g/mm2 or mg/cm2
head(sample_info2)
```

```
## # A tibble: 6 x 5
##   Plant_Species         Species_Code Plot  Narea_mg_mm2 Narea_g_m2
##   <chr>                 <chr>        <chr>        <dbl>      <dbl>
## 1 Arrhenatherum elatius Arrela       DC1        0.0126       1.26
## 2 Bromus sterilis       Broste       DC1        0.00682      0.682
## 3 Jacobaea vulgaris     Jacvul       DC1        0.0102       1.02
## 4 Rubus caesius         Rubcae       DC1        0.0121       1.21
## 5 Arrhenatherum elatius Arrela       DC2        0.0117       1.17
## 6 Crepis capillaris     Creves       DC2        0.00877      0.877
```

```
plsr_data <- data.frame(sample_info2,Spectra)
rm(sample_info,sample_info2,Spectra)
```

```
#### End user needs to do what's appropriate for their data.
#### This may be an iterative process.
# Keep only complete rows of inVar and spec data before fitting
plsr_data <- plsr_data[complete.cases(plsr_data[,names(plsr_data) %in%
                                                  c(inVar,paste0("Wave_",wv))]),]
```

**Example data cleaning.**

**Create cal/val datasets**

```
### Create cal/val datasets
## Make a stratified random sampling in the strata USDA_Species_Code and Domain

method <- "dplyr" #base/dplyr
# base R - a bit slow
# dplyr - much faster
split_data <- spectratrait::create_data_split(dataset=plsr_data, approach=method, split_seed=1245565,
                                prop=0.8, group_variables="Species_Code")
names(split_data)
```

```
## [1] "cal_data" "val_data"
```

```
cal.plsr.data <- split_data$cal_data
head(cal.plsr.data)[1:8]
```

```
##        Plant_Species Species_Code Plot Narea_mg_mm2 Narea_g_m2 Wave_500
## 1 Ammophila arenaria       Ammare  ZC3   0.03240495   3.240495 0.130885
## 2 Ammophila arenaria       Ammare  MC2   0.02806279   2.806279 0.135785
## 3 Ammophila arenaria       Ammare  ZC1   0.02041612   2.041612 0.147665
## 4 Ammophila arenaria       Ammare  MC1   0.02426549   2.426549 0.142765
## 5 Ammophila arenaria       Ammare  WC3   0.02807281   2.807281 0.151750
## 6 Ammophila arenaria       Ammare  WR3   0.02286678   2.286678 0.150850
##   Wave_501 Wave_502
## 1  0.13175 0.132750
## 2  0.13685 0.138150
## 3  0.14910 0.150330
## 4  0.14390 0.145200
## 5  0.15275 0.154150
## 6  0.15185 0.152815
```

```
val.plsr.data <- split_data$val_data
head(val.plsr.data)[1:8]
```

```
##            Plant_Species Species_Code Plot Narea_mg_mm2 Narea_g_m2   Wave_500
## 1  Arrhenatherum elatius       Arrela  DC1   0.01261440   1.261440 0.07066700
## 4           Rubus caesius       Rubcae  DC1   0.01208978   1.208978 0.04144907
## 8       Jacobaea vulgaris       Jacvul  DC2   0.01185197   1.185197 0.05563100
## 11         Carex arenaria       Carare  DC3   0.02103830   2.103830 0.11588500
## 14      Jacobaea vulgaris       Jacvul  DC3   0.01121247   1.121247 0.06029327
## 19 Oenothera glazioviana       Oengla  DC4   0.01444293   1.444293 0.07391700
##      Wave_501  Wave_502
```

```
## 1  0.07160000 0.0725330
## 4  0.04197333 0.0426356
## 8  0.05622143 0.0569690
## 11 0.11705000 0.1184500
## 14 0.06112000 0.0620312
## 19 0.07515000 0.0765500
```

```
rm(split_data)

# Datasets:
print(paste("Cal observations: ",dim(cal.plsr.data)[1],sep=""))
```

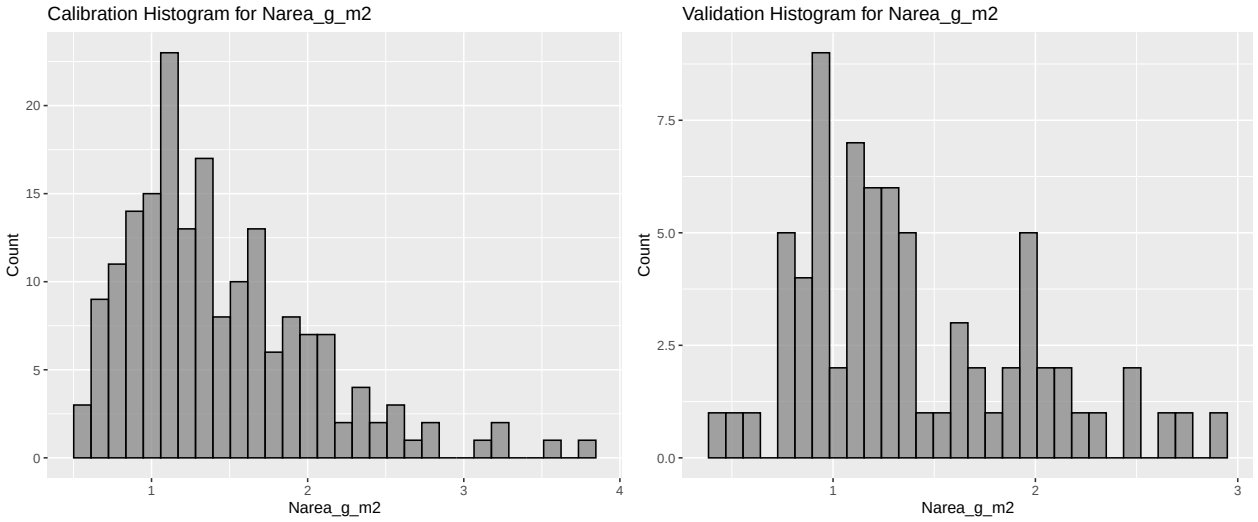
```
## [1] "Cal observations: 183"
```

```
print(paste("Val observations: ",dim(val.plsr.data)[1],sep=""))
```

```
## [1] "Val observations: 73"
```

```
cal_hist_plot <- ggplot(data = cal.plsr.data,
                        aes(x = cal.plsr.data[,paste0(inVar)])) +
  geom_histogram(fill=I("grey50"),col=I("black"),alpha=I(.7)) +
  labs(title=paste0("Calibration Histogram for ",inVar), x = paste0(inVar),
       y = "Count")
val_hist_plot <- ggplot(data = val.plsr.data,
                        aes(x = val.plsr.data[,paste0(inVar)])) +
  geom_histogram(fill=I("grey50"),col=I("black"),alpha=I(.7)) +
  labs(title=paste0("Validation Histogram for ",inVar), x = paste0(inVar),
       y = "Count")
histograms <- grid.arrange(cal_hist_plot, val_hist_plot, ncol=2)
```

```
## `stat_bin()` using `bins = 30`. Pick better value with `binwidth`.
## `stat_bin()` using `bins = 30`. Pick better value with `binwidth`.
```




```
ggsave(filename = file.path(outdir,paste0(inVar,"_Cal_Val_Histograms.png")), plot = histograms,
       device="png", width = 30,
       height = 12, units = "cm",
       dpi = 300)
# output cal/val data
write.csv(cal.plsr.data,file=file.path(outdir,paste0(inVar,'_Cal_PLSR_Dataset.csv')),
          row.names=FALSE)
```

```
write.csv(val.plsr.data,file=file.path(outdir,paste0(inVar,'_Val_PLSR_Dataset.csv')),
          row.names=FALSE)
```

### Create calibration and validation PLSR datasets

```
### Format PLSR data for model fitting
cal_spec <- as.matrix(cal.plsr.data[, which(names(cal.plsr.data) %in% paste0("Wave_",wv))])
cal.plsr.data <- data.frame(cal.plsr.data[, which(names(cal.plsr.data) %notin% paste0("Wave_",wv))],
                            Spectra=I(cal_spec))
head(cal.plsr.data)[1:5]
```

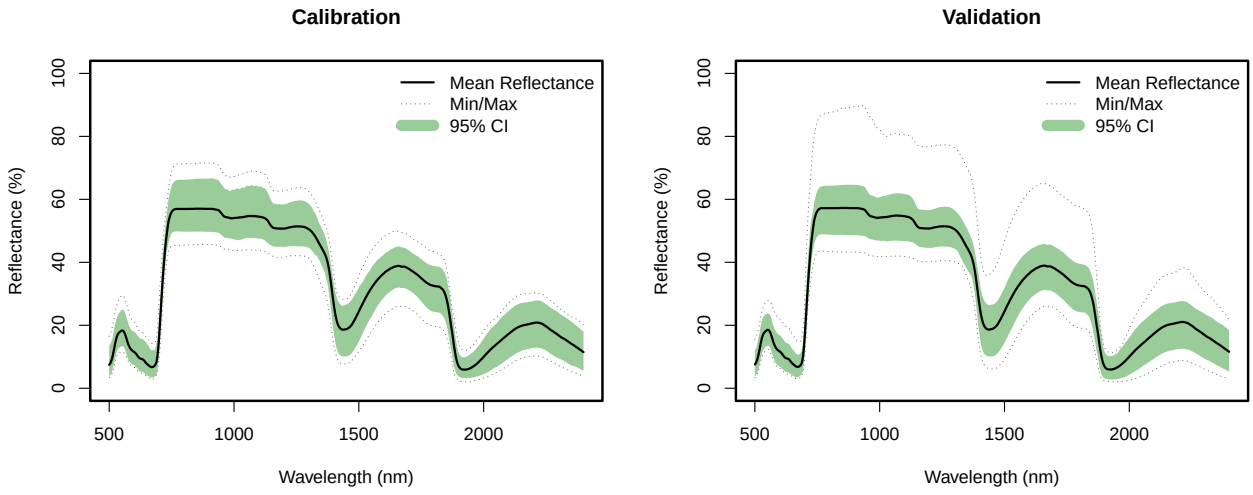
```
##        Plant_Species Species_Code Plot Narea_mg_mm2 Narea_g_m2
## 1 Ammophila arenaria       Ammare  ZC3   0.03240495   3.240495
## 2 Ammophila arenaria       Ammare  MC2   0.02806279   2.806279
## 3 Ammophila arenaria       Ammare  ZC1   0.02041612   2.041612
## 4 Ammophila arenaria       Ammare  MC1   0.02426549   2.426549
## 5 Ammophila arenaria       Ammare  WC3   0.02807281   2.807281
## 6 Ammophila arenaria       Ammare  WR3   0.02286678   2.286678
```

```
val_spec <- as.matrix(val.plsr.data[, which(names(val.plsr.data) %in% paste0("Wave_",wv))])
val.plsr.data <- data.frame(val.plsr.data[, which(names(val.plsr.data) %notin% paste0("Wave_",wv))],
                            Spectra=I(val_spec))
head(val.plsr.data)[1:5]
```

```
##            Plant_Species Species_Code Plot Narea_mg_mm2 Narea_g_m2
## 1  Arrhenatherum elatius       Arrela  DC1   0.01261440   1.261440
## 4          Rubus caesius       Rubcae  DC1   0.01208978   1.208978
## 8      Jacobaea vulgaris       Jacvul  DC2   0.01185197   1.185197
## 11        Carex arenaria       Carare  DC3   0.02103830   2.103830
## 14     Jacobaea vulgaris       Jacvul  DC3   0.01121247   1.121247
## 19 Oenothera glazioviana       Oengla  DC4   0.01444293   1.444293
```

### plot cal and val spectra

```
par(mfrow=c(1,2)) # B, L, T, R
spectratrait::f.plot.spec(Z=cal.plsr.data$Spectra,wv=wv,plot_label="Calibration")
spectratrait::f.plot.spec(Z=val.plsr.data$Spectra,wv=wv,plot_label="Validation")
```



```
dev.copy(png,file.path(outdir,paste0(inVar,'_Cal_Val_Spectra.png')),
         height=2500,width=4900, res=340)
```

```
## quartz_off_screen
##                 3
```

```
dev.off();
```

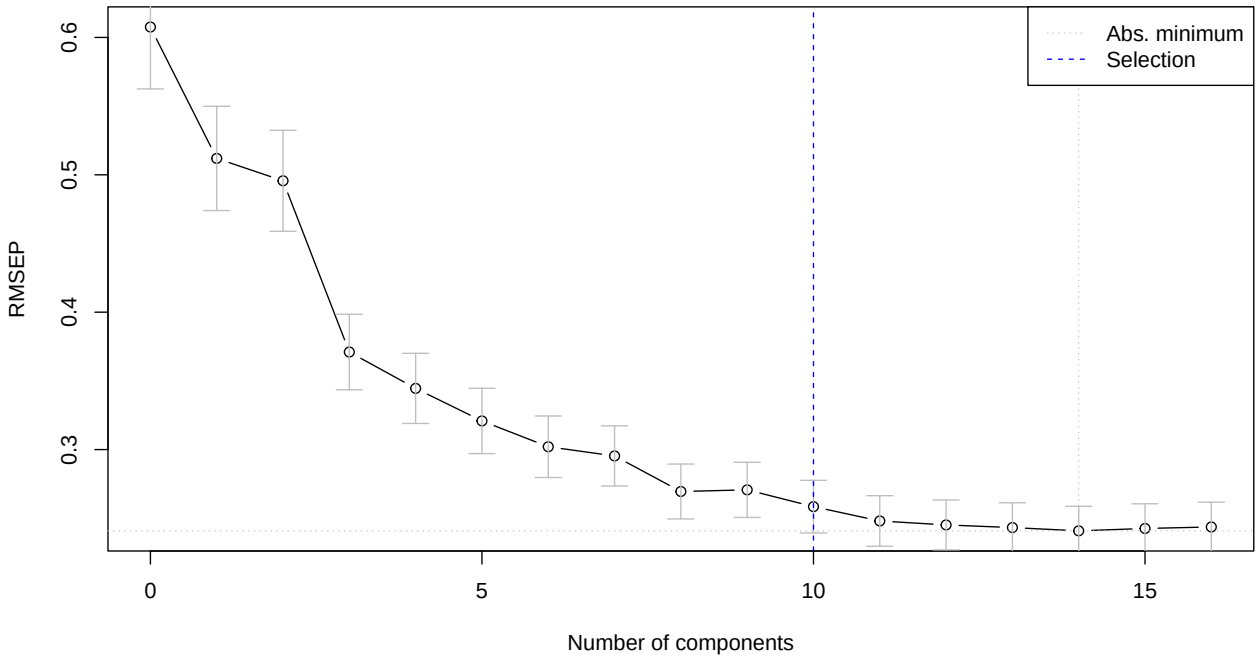
```
## pdf
##   2
```

```
par(mfrow=c(1,1))
```

### Use permutation to determine optimal number of components

```
### Use permutation to determine the optimal number of components
if(grepl("Windows", sessionInfo()$running)){
  pls.options(parallel = NULL)
} else {
  pls.options(parallel = parallel::detectCores()-1)
}

method <- "pls" #pls, firstPlateau, firstMin
random_seed <- 1245565
seg <- 50
maxComps <- 16
iterations <- 80
prop <- 0.70
if (method=="pls") {
  # pls package approach - faster but estimates more components....
  nComps <- spectratrait::find_optimal_components(dataset=cal.plsr.data, targetVariable=inVar,
                                                  method=method,
                                                  maxComps=maxComps, seg=seg,
                                                  random_seed=random_seed)
  print(paste0("*** Optimal number of components: ", nComps))
} else {
  nComps <- spectratrait::find_optimal_components(dataset=cal.plsr.data, targetVariable=inVar,
                                                  method=method,
                                                  maxComps=maxComps, iterations=iterations,
                                                  seg=seg, prop=prop,
                                                  random_seed=random_seed)
}
```

```
## [1] "*** Identifying optimal number of PLSR components ***"
## [1] "*** Running PLS permutation test ***"
```




```
## [1] "*** Optimal number of components: 10"
```

```
dev.copy(png,file.path(outdir,paste0(paste0(inVar,"_PLSR_Component_Selection.png"))),
         height=2800, width=3400,  res=340)
```

```
## quartz_off_screen
##                 3
```

```
dev.off();
```

```
## pdf
##   2
```

### Fit final model

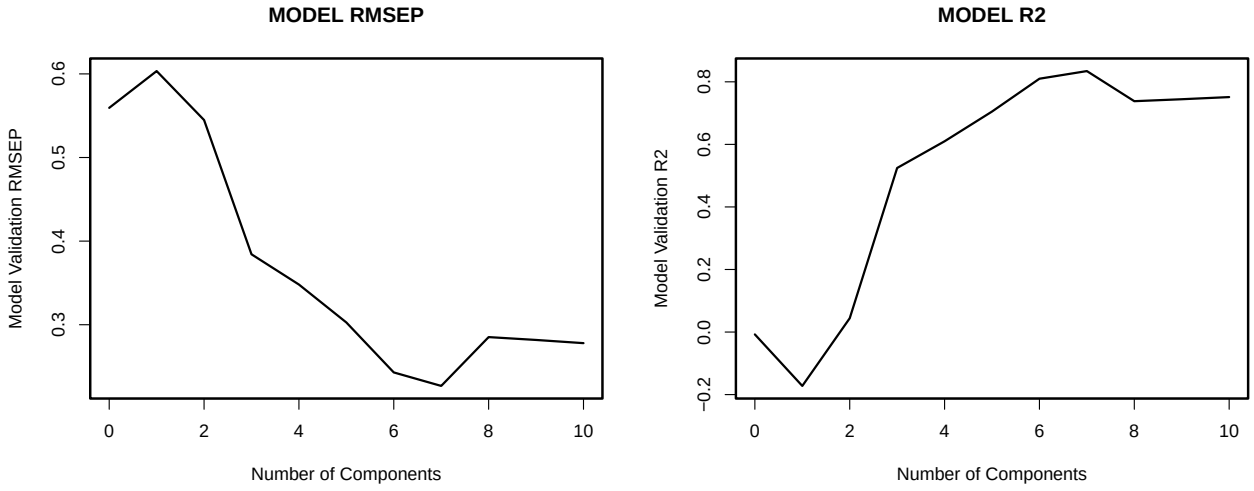
```
plsr.out <- plsr(as.formula(paste(inVar,"~","Spectra")),scale=FALSE,ncomp=nComps,validation="LOO",
                 trace=FALSE,data=cal.plsr.data)
fit <- plsr.out$fitted.values[,1,nComps]
pls.options(parallel = NULL)

# External validation fit stats
par(mfrow=c(1,2)) # B, L, T, R
pls::RMSEP(plsr.out, newdata = val.plsr.data)
```

```
## (Intercept)      1 comps      2 comps      3 comps      4 comps      5 comps
##      0.5594       0.6034       0.5448       0.3842       0.3481       0.3027
##     6 comps      7 comps      8 comps      9 comps     10 comps
##      0.2429       0.2268       0.2852       0.2818       0.2780
```

```
plot(pls::RMSEP(plsr.out,estimate=c("test"),newdata = val.plsr.data), main="MODEL RMSEP",
     xlab="Number of Components",ylab="Model Validation RMSEP",lty=1,col="black",cex=1.5,lwd=2)
box(lwd=2.2)

pls::R2(plsr.out, newdata = val.plsr.data)
```

```
## (Intercept)      1 comps      2 comps      3 comps      4 comps      5 comps
##   -0.007544    -0.172296     0.044153     0.524579     0.609920     0.704963
##     6 comps      7 comps      8 comps      9 comps     10 comps
##    0.809962     0.834383     0.738093     0.744325     0.751224
```

```
plot(pls::R2(plsr.out,estimate=c("test"),newdata = val.plsr.data), main="MODEL R2",
     xlab="Number of Components",ylab="Model Validation R2",lty=1,col="black",cex=1.5,lwd=2)
box(lwd=2.2)
```




```
dev.copy(png,file.path(outdir,paste0(paste0(inVar,"_Validation_RMSEP_R2_by_Component.png"))),
         height=2800, width=4800,  res=340)
```

```
## quartz_off_screen
##                 3
```

```
dev.off();
```

```
## pdf
##   2
```

```
par(opar)
```

### PLSR fit observed vs. predicted plot data

```
#calibration
cal.plsr.output <- data.frame(cal.plsr.data[, which(names(cal.plsr.data) %notin% "Spectra")],
                              PLSR_Predicted=fit,
                              PLSR_CV_Predicted=as.vector(plsr.out$validation$pred[,,nComps]))
cal.plsr.output <- cal.plsr.output %>%
  mutate(PLSR_CV_Residuals = PLSR_CV_Predicted-get(inVar))
head(cal.plsr.output)
```

```
##        Plant_Species Species_Code Plot Narea_mg_mm2 Narea_g_m2 PLSR_Predicted
## 1 Ammophila arenaria       Ammare  ZC3   0.03240495   3.240495       2.672029
## 2 Ammophila arenaria       Ammare  MC2   0.02806279   2.806279       2.651863
## 3 Ammophila arenaria       Ammare  ZC1   0.02041612   2.041612       2.178056
## 4 Ammophila arenaria       Ammare  MC1   0.02426549   2.426549       2.412013
## 5 Ammophila arenaria       Ammare  WC3   0.02807281   2.807281       2.452711
## 6 Ammophila arenaria       Ammare  WR3   0.02286678   2.286678       2.792340
##   PLSR_CV_Predicted PLSR_CV_Residuals
## 1          2.598245      -0.642250440
```

```
## 2          2.652066      -0.154212969
## 3          2.200588       0.158975634
## 4          2.435784       0.009234491
## 5          2.384049      -0.423231444
## 6          2.943186       0.656508493
```

```
cal.R2 <- round(pls::R2(plsr.out,intercept=F)[[1]][nComps],2)
cal.RMSEP <- round(sqrt(mean(cal.plsr.output$PLSR_CV_Residuals^2)),2)

val.plsr.output <- data.frame(val.plsr.data[, which(names(val.plsr.data) %notin% "Spectra")],
                              PLSR_Predicted=as.vector(predict(plsr.out,
                                                               newdata = val.plsr.data,
                                                               ncomp=nComps, type="response")[,,1]))
val.plsr.output <- val.plsr.output %>%
  mutate(PLSR_Residuals = PLSR_Predicted-get(inVar))
head(val.plsr.output)
```

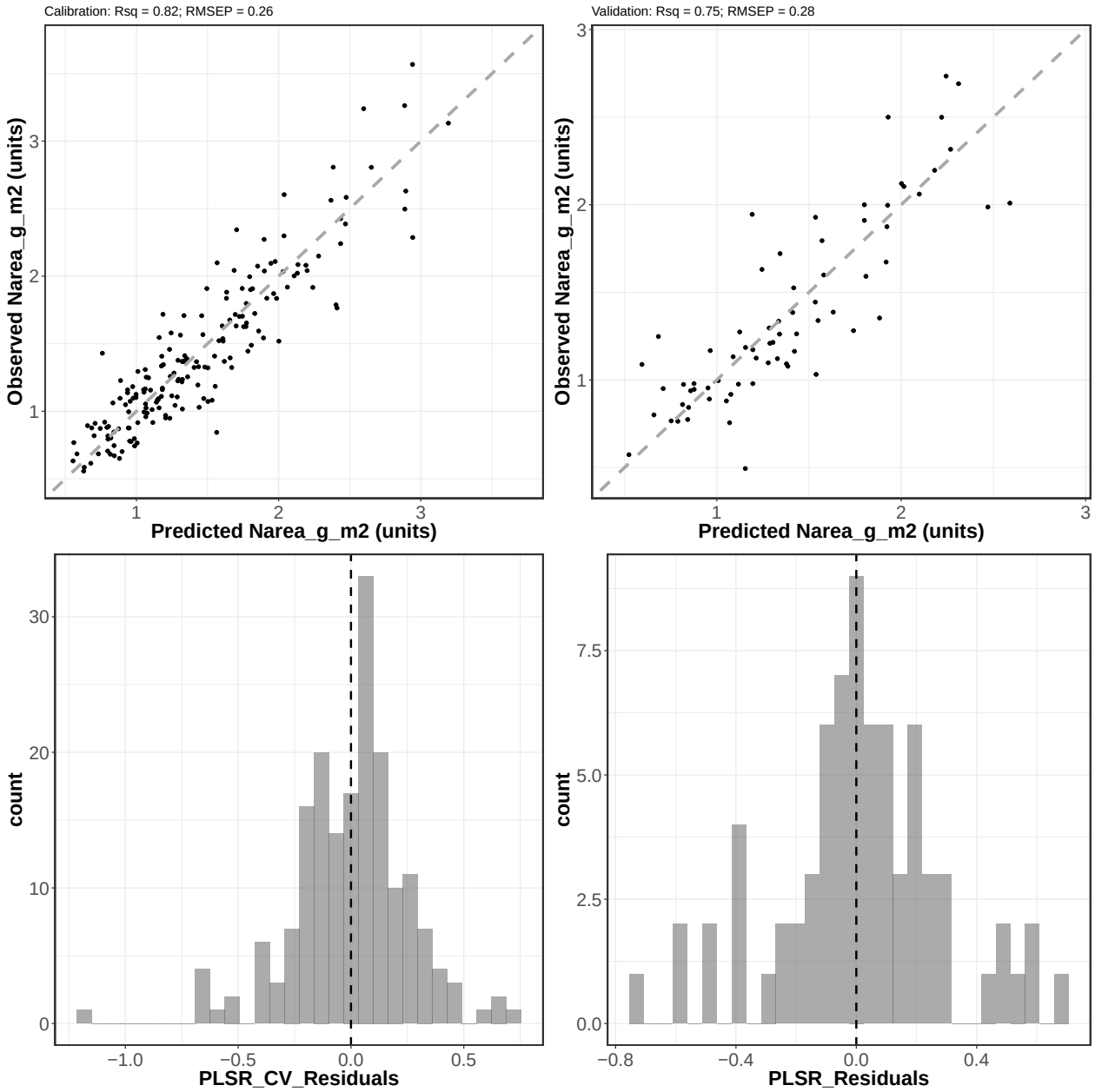
```
##            Plant_Species Species_Code Plot Narea_mg_mm2 Narea_g_m2
## 1  Arrhenatherum elatius       Arrela  DC1   0.01261440   1.261440
## 4           Rubus caesius       Rubcae  DC1   0.01208978   1.208978
## 8       Jacobaea vulgaris       Jacvul  DC2   0.01185197   1.185197
## 11         Carex arenaria       Carare  DC3   0.02103830   2.103830
## 14      Jacobaea vulgaris       Jacvul  DC3   0.01121247   1.121247
## 19 Oenothera glazioviana       Oengla  DC4   0.01444293   1.444293
##    PLSR_Predicted PLSR_Residuals
## 1        1.340135     0.07869548
## 4        1.288026     0.07904830
## 8        1.155840    -0.02935675
## 11       2.014712    -0.08911757
## 14       1.328742     0.20749565
## 19       1.534162     0.08986811
```

```
val.R2 <- round(pls::R2(plsr.out,newdata=val.plsr.data,intercept=F)[[1]][nComps],2)
val.RMSEP <- round(sqrt(mean(val.plsr.output$PLSR_Residuals^2)),2)

rng_quant <- quantile(cal.plsr.output[,inVar], probs = c(0.001, 0.999))
cal_scatter_plot <- ggplot(cal.plsr.output, aes(x=PLSR_CV_Predicted, y=get(inVar))) +
  theme_bw() + geom_point() + geom_abline(intercept = 0, slope = 1, color="dark grey",
                                          linetype="dashed", linewidth=1.5) +
  xlim(rng_quant[1], rng_quant[2]) +
  ylim(rng_quant[1], rng_quant[2]) +
  labs(x=paste0("Predicted ", paste(inVar), " (units)"),
       y=paste0("Observed ", paste(inVar), " (units)"),
       title=paste0("Calibration: ", paste0("Rsq = ", cal.R2), "; ", paste0("RMSEP = ",
                                                                            cal.RMSEP))) +
  theme(axis.text=element_text(size=18), legend.position="none",
        axis.title=element_text(size=20, face="bold"),
        axis.text.x = element_text(angle = 0,vjust = 0.5),
        panel.border = element_rect(linetype = "solid", fill = NA, linewidth=1.5))

cal_resid_histogram <- ggplot(cal.plsr.output, aes(x=PLSR_CV_Residuals)) +
  geom_histogram(alpha=.5, position="identity") +
  geom_vline(xintercept = 0, color="black",
             linetype="dashed", linewidth=1) + theme_bw() +
  theme(axis.text=element_text(size=18), legend.position="none",
```

```
        axis.title=element_text(size=20, face="bold"),
        axis.text.x = element_text(angle = 0,vjust = 0.5),
        panel.border = element_rect(linetype = "solid", fill = NA, linewidth=1.5))

rng_quant <- quantile(val.plsr.output[,inVar], probs = c(0.001, 0.999))
val_scatter_plot <- ggplot(val.plsr.output, aes(x=PLSR_Predicted, y=get(inVar))) +
  theme_bw() + geom_point() + geom_abline(intercept = 0, slope = 1, color="dark grey",
                                          linetype="dashed", linewidth=1.5) +
  xlim(rng_quant[1], rng_quant[2]) +
  ylim(rng_quant[1], rng_quant[2]) +
  labs(x=paste0("Predicted ", paste(inVar), " (units)"),
       y=paste0("Observed ", paste(inVar), " (units)"),
       title=paste0("Validation: ", paste0("Rsq = ", val.R2), "; ", paste0("RMSEP = ",
                                                                           val.RMSEP))) +
  theme(axis.text=element_text(size=18), legend.position="none",
        axis.title=element_text(size=20, face="bold"),
        axis.text.x = element_text(angle = 0,vjust = 0.5),
        panel.border = element_rect(linetype = "solid", fill = NA, linewidth=1.5))

val_resid_histogram <- ggplot(val.plsr.output, aes(x=PLSR_Residuals)) +
  geom_histogram(alpha=.5, position="identity") +
  geom_vline(xintercept = 0, color="black",
             linetype="dashed", linewidth=1) + theme_bw() +
  theme(axis.text=element_text(size=18), legend.position="none",
        axis.title=element_text(size=20, face="bold"),
        axis.text.x = element_text(angle = 0,vjust = 0.5),
        panel.border = element_rect(linetype = "solid", fill = NA, linewidth=1.5))

# plot cal/val side-by-side
scatterplots <- grid.arrange(cal_scatter_plot, val_scatter_plot, cal_resid_histogram,
                             val_resid_histogram, nrow=2,ncol=2)
```

```
## Warning: Removed 2 rows containing missing values or values outside the scale range
## (`geom_point()`).
## Removed 2 rows containing missing values or values outside the scale range
## (`geom_point()`).
```

```
## `stat_bin()` using `bins = 30`. Pick better value with `binwidth`.
## `stat_bin()` using `bins = 30`. Pick better value with `binwidth`.
```

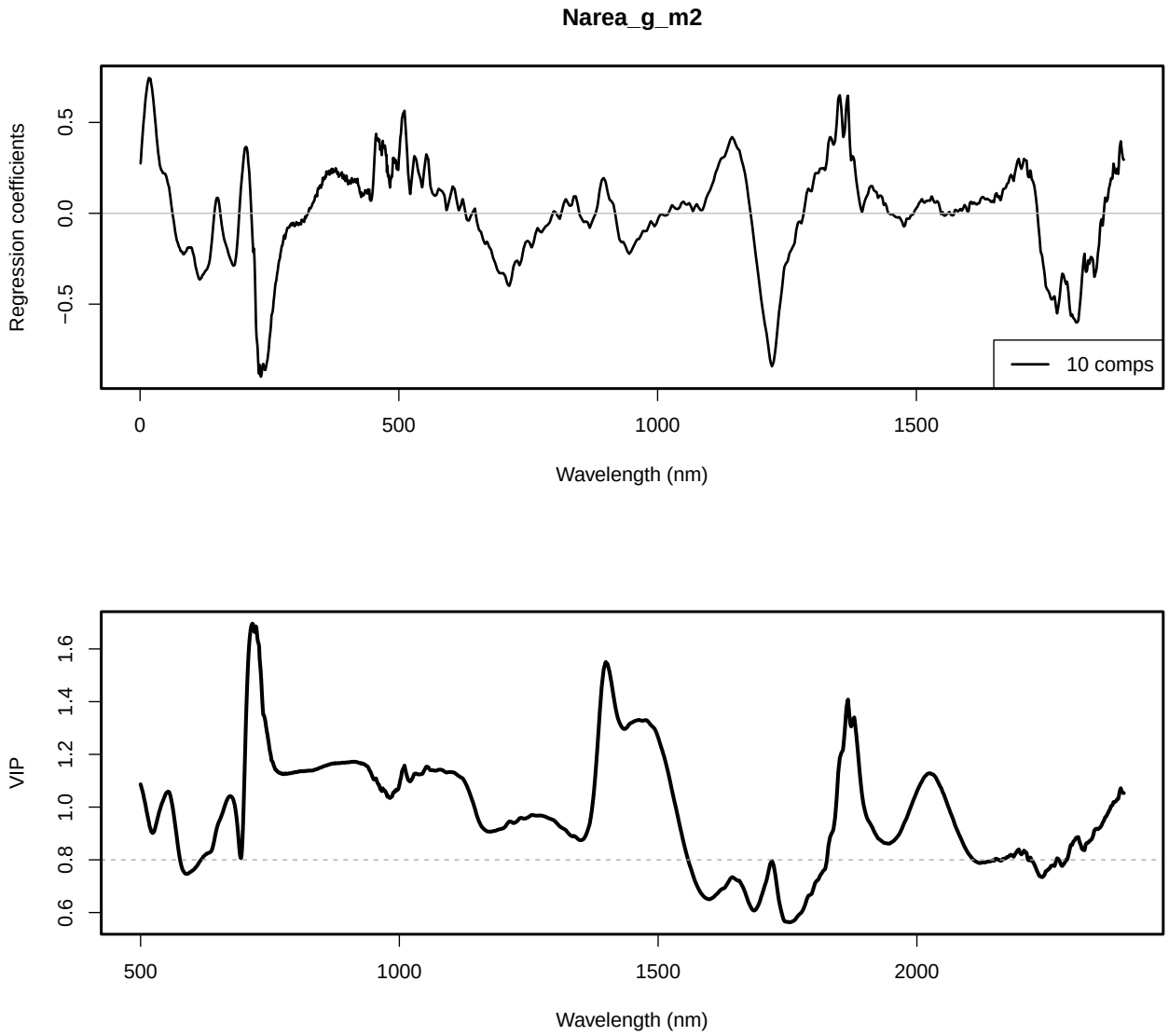



```
ggsave(filename = file.path(outdir,paste0(inVar,"_Cal_Val_Scatterplots.png")),
       plot = scatterplots, device="png",
       width = 32,
       height = 30, units = "cm",
       dpi = 300)
```

### Generate Coefficient and VIP plots

```
vips <- spectratrait::VIP(plsr.out)[nComps,]
par(mfrow=c(2,1))
plot(plsr.out, plottype = "coef",xlab="Wavelength (nm)",
     ylab="Regression coefficients",legendpos = "bottomright",
     ncomp=nComps,lwd=2)
box(lwd=2.2)
```

```
plot(seq(Start.wave,End.wave,1),vips,xlab="Wavelength (nm)",ylab="VIP",cex=0.01)
lines(seq(Start.wave,End.wave,1),vips,lwd=3)
abline(h=0.8,lty=2,col="dark grey")
box(lwd=2.2)
```




```
dev.copy(png,file.path(outdir,paste0(inVar,'_Coefficient_VIP_plot.png')),
         height=3100, width=4100, res=340)
```

```
## quartz_off_screen
##                 3
```

```
dev.off();
```

```
## pdf
##   2
```

### Bootstrap validation

```
if(grepl("Windows", sessionInfo()$running)){
  pls.options(parallel =NULL)
} else {
  pls.options(parallel = parallel::detectCores()-1)
}

### PLSR bootstrap permutation uncertainty analysis
iterations <- 500    # how many permutation iterations to run
prop <- 0.70          # fraction of training data to keep for each iteration
plsr_permutation <- spectratrait::pls_permutation(dataset=cal.plsr.data, targetVariable=inVar,
                                                  maxComps=nComps,
                                                  iterations=iterations, prop=prop,
                                                  verbose = FALSE)
```

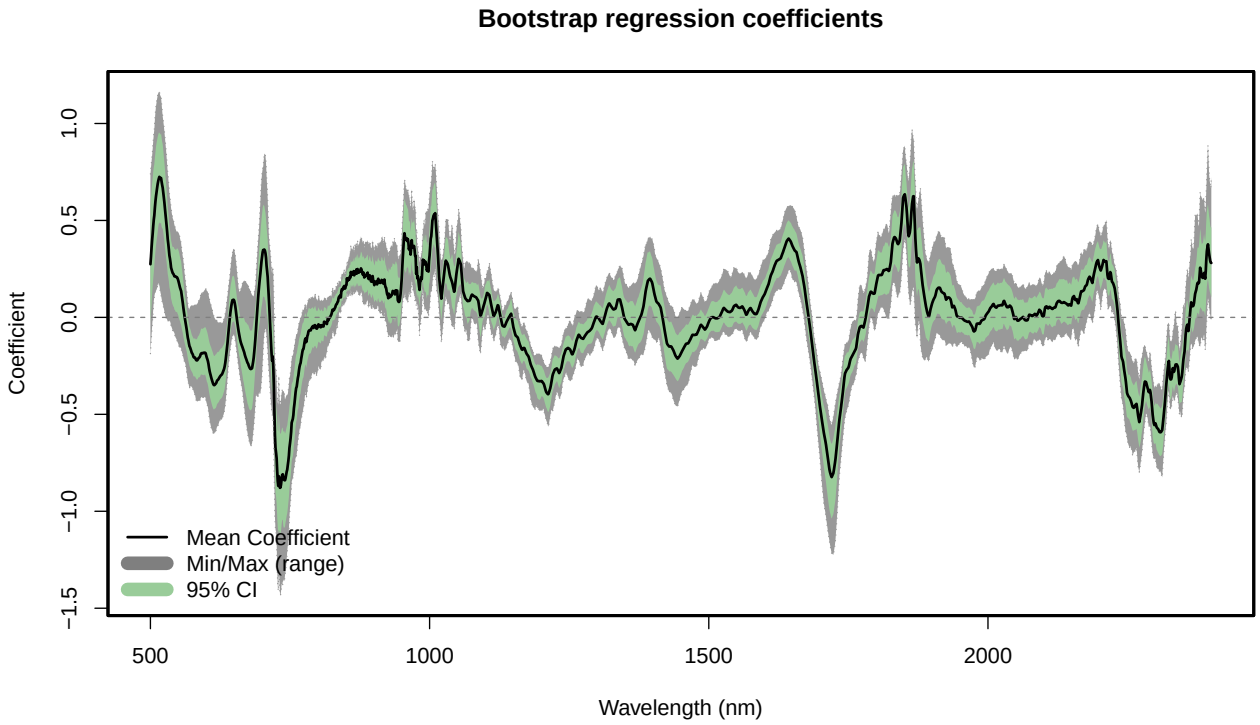
```
## [1] "*** Running permutation test.  Please hang tight, this can take awhile ***"
## [1] "Options:"
## [1] "Max Components: 10 Iterations: 500 Data Proportion (percent): 70"
## [1] "*** Providing PRESS and coefficient array output ***"
```

```
bootstrap_intercept <- plsr_permutation$coef_array[1,,nComps]
bootstrap_coef <- plsr_permutation$coef_array[2:length(plsr_permutation$coef_array[,1,nComps]),
                                              ,nComps]
rm(plsr_permutation)

# apply coefficients to left-out validation data
interval <- c(0.025,0.975)
Bootstrap_Pred <- val.plsr.data$Spectra %*% bootstrap_coef +
  matrix(rep(bootstrap_intercept, length(val.plsr.data[,inVar])), byrow=TRUE,
         ncol=length(bootstrap_intercept))
Interval_Conf <- apply(X = Bootstrap_Pred, MARGIN = 1, FUN = quantile,
                       probs=c(interval[1], interval[2]))
sd_mean <- apply(X = Bootstrap_Pred, MARGIN = 1, FUN = sd)
sd_res <- sd(val.plsr.output$PLSR_Residuals)
sd_tot <- sqrt(sd_mean^2+sd_res^2)
val.plsr.output$LCI <- Interval_Conf[1,]
val.plsr.output$UCI <- Interval_Conf[2,]
val.plsr.output$LPI <- val.plsr.output$PLSR_Predicted-1.96*sd_tot
val.plsr.output$UPI <- val.plsr.output$PLSR_Predicted+1.96*sd_tot
head(val.plsr.output)
```

```
##            Plant_Species Species_Code Plot Narea_mg_mm2 Narea_g_m2
## 1  Arrhenatherum elatius       Arrela  DC1   0.01261440   1.261440
## 4           Rubus caesius       Rubcae  DC1   0.01208978   1.208978
## 8       Jacobaea vulgaris       Jacvul  DC2   0.01185197   1.185197
## 11         Carex arenaria       Carare  DC3   0.02103830   2.103830
## 14      Jacobaea vulgaris       Jacvul  DC3   0.01121247   1.121247
## 19 Oenothera glazioviana       Oengla  DC4   0.01444293   1.444293
##    PLSR_Predicted PLSR_Residuals      LCI      UCI       LPI      UPI
## 1        1.340135     0.07869548 1.226834 1.429328 0.7824784 1.897793
## 4        1.288026     0.07904830 1.182535 1.382687 0.7308093 1.845242
## 8        1.155840    -0.02935675 1.094629 1.236994 0.6033307 1.708349
## 11       2.014712    -0.08911757 1.887670 2.098661 1.4570138 2.572411
## 14       1.328742     0.20749565 1.278652 1.359115 0.7791767 1.878308
## 19       1.534162     0.08986811 1.437618 1.642761 0.9768182 2.091505
```

### Jackknife coefficient plot

```
# Bootstrap regression coefficient plot
spectratrait::f.plot.coef(Z = t(bootstrap_coef), wv = wv,
            plot_label="Bootstrap regression coefficients",position = 'bottomleft')
abline(h=0,lty=2,col="grey50")
box(lwd=2.2)
```




```
dev.copy(png,file.path(outdir,paste0(inVar,'_Bootstrap_Regression_Coefficients.png')),
         height=2100, width=3800, res=340)
```

```
## quartz_off_screen
##                 3
```

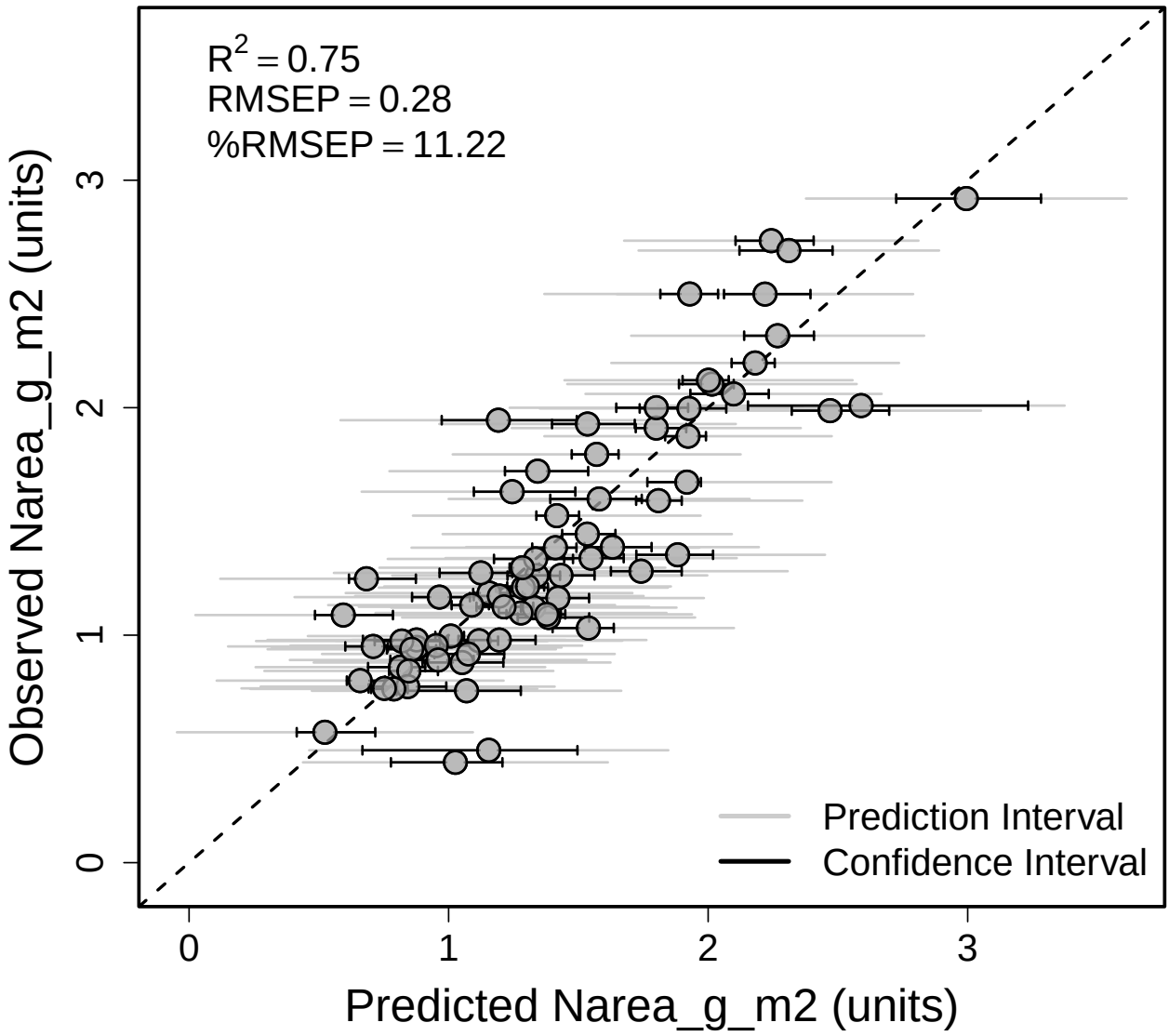
```
dev.off();
```

```
## pdf
##   2
```

### Bootstrap validation plot

```
rmsep_percrmsep <- spectratrait::percent_rmse(plsr_dataset = val.plsr.output,
                                              inVar = inVar,
                                              residuals = val.plsr.output$PLSR_Residuals,
                                              range="full")
RMSEP <- rmsep_percrmsep$rmse
perc_RMSEP <- rmsep_percrmsep$perc_rmse
r2 <- round(pls::R2(plsr.out, newdata = val.plsr.data,intercept=F)$val[nComps],2)
expr <- vector("expression", 3)
expr[[1]] <- bquote(R^2==.(r2))
expr[[2]] <- bquote(RMSEP==.(round(RMSEP,2)))
expr[[3]] <- bquote("%RMSEP"==.(round(perc_RMSEP,2)))
```

```
rng_vals <- c(min(val.plsr.output$LPI), max(val.plsr.output$UPI))
par(mfrow=c(1,1), mar=c(4.2,5.3,1,0.4), oma=c(0, 0.1, 0, 0.2))
plotrix::plotCI(val.plsr.output$PLSR_Predicted,val.plsr.output[,inVar],
       li=val.plsr.output$LPI, ui=val.plsr.output$UPI, gap=0.009,sfrac=0.000,
       lwd=1.6, xlim=c(rng_vals[1], rng_vals[2]), ylim=c(rng_vals[1], rng_vals[2]),
       err="x", pch=21, col="black", pt.bg=scales::alpha("grey70",0.7), scol="grey80",
       cex=2, xlab=paste0("Predicted ", paste(inVar), " (units)"),
       ylab=paste0("Observed ", paste(inVar), " (units)"),
       cex.axis=1.5,cex.lab=1.8)
abline(0,1,lty=2,lw=2)
plotrix::plotCI(val.plsr.output$PLSR_Predicted,val.plsr.output[,inVar],
       li=val.plsr.output$LCI, ui=val.plsr.output$UCI, gap=0.009,sfrac=0.004,
       lwd=1.6, xlim=c(rng_vals[1], rng_vals[2]), ylim=c(rng_vals[1], rng_vals[2]),
       err="x", pch=21, col="black", pt.bg=scales::alpha("grey70",0.7), scol="black",
       cex=2, xlab=paste0("Predicted ", paste(inVar), " (units)"),
       ylab=paste0("Observed ", paste(inVar), " (units)"),
       cex.axis=1.5,cex.lab=1.8, add=T)
legend("topleft", legend=expr, bty="n", cex=1.5)
legend("bottomright", legend=c("Prediction Interval","Confidence Interval"),
       lty=c(1,1), col = c("grey80","black"), lwd=3, bty="n", cex=1.5)
box(lwd=2.2)
```




```
dev.copy(png,file.path(outdir,paste0(inVar,"_PLSR_Validation_Scatterplot.png")),
         height=2800, width=3200,  res=340)
```

```
## quartz_off_screen
##                 3
```

```
dev.off();
```

```
## pdf
##   2
```

**Output bootstrap results**

```
# Bootstrap Coefficients
out.jk.coefs <- data.frame(Iteration=seq(1,length(bootstrap_intercept),1),
                           Intercept=bootstrap_intercept,t(bootstrap_coef))
names(out.jk.coefs) <- c("Iteration","Intercept",paste0("Wave_",wv))
head(out.jk.coefs)[1:6]
```

```
##   Iteration   Intercept   Wave_500  Wave_501  Wave_502  Wave_503
```

```
## 1         1 -0.13686765 0.29141489 0.3287594 0.3654695 0.3999712
## 2         2 -0.17612080 0.24114488 0.2827001 0.3182992 0.3552505
## 3         3  0.34135463 0.21939317 0.2562451 0.2984578 0.3330333
## 4         4  0.01511507 0.09404839 0.1299058 0.1792805 0.2240698
## 5         5  0.06136605 0.12835311 0.1662322 0.2116938 0.2539193
## 6         6  0.10925409 0.28154095 0.3107509 0.3539714 0.3932620
```

```
write.csv(out.jk.coefs,file=file.path(outdir,paste0(inVar,
                                                    '_Bootstrap_PLSR_Coefficients.csv')),
          row.names=FALSE)
```

### Create core PLSR outputs

```
print(paste("Output directory: ", outdir))
```

```
## [1] "Output directory:  /var/folders/th/fpt_z3417gn8xgply92pvy6r0000gq/T//Rtmp41ruG8"
```

```
# Observed versus predicted
write.csv(cal.plsr.output,file=file.path(outdir,
                                         paste0(inVar,'_Observed_PLSR_CV_Pred_',
                                                nComps,'comp.csv')),
          row.names=FALSE)

# Validation data
write.csv(val.plsr.output,file=file.path(outdir,
                                         paste0(inVar,'_Validation_PLSR_Pred_',
                                                nComps,'comp.csv')),
          row.names=FALSE)

# Model coefficients
coefs <- coef(plsr.out,ncomp=nComps,intercept=TRUE)
write.csv(coefs,file=file.path(outdir,
                               paste0(inVar,'_PLSR_Coefficients_',
                                      nComps,'comp.csv')),
          row.names=TRUE)

# PLSR VIP
write.csv(vips,file=file.path(outdir,
                              paste0(inVar,'_PLSR_VIPs_',
                                     nComps,'comp.csv')))
```

### Confirm files were written to temp space

```
print("**** PLSR output files: ")
```

```
## [1] "**** PLSR output files: "
```

```
print(list.files(outdir)[grep(pattern = inVar, list.files(outdir))])
```

```
##  [1] "Narea_g_m2_Bootstrap_PLSR_Coefficients.csv"
##  [2] "Narea_g_m2_Bootstrap_Regression_Coefficients.png"
##  [3] "Narea_g_m2_Cal_PLSR_Dataset.csv"
##  [4] "Narea_g_m2_Cal_Val_Histograms.png"
##  [5] "Narea_g_m2_Cal_Val_Scatterplots.png"
##  [6] "Narea_g_m2_Cal_Val_Spectra.png"
```

```
##  [7] "Narea_g_m2_Coefficient_VIP_plot.png"
##  [8] "Narea_g_m2_Observed_PLSR_CV_Pred_10comp.csv"
##  [9] "Narea_g_m2_PLSR_Coefficients_10comp.csv"
## [10] "Narea_g_m2_PLSR_Component_Selection.png"
## [11] "Narea_g_m2_PLSR_Validation_Scatterplot.png"
## [12] "Narea_g_m2_PLSR_VIPs_10comp.csv"
## [13] "Narea_g_m2_Val_PLSR_Dataset.csv"
## [14] "Narea_g_m2_Validation_PLSR_Pred_10comp.csv"
## [15] "Narea_g_m2_Validation_RMSEP_R2_by_Component.png"
```