Spectra-trait PLSR example using leaf-level spectra and leaf mass per area (LMA) data from 36 species growing in Rosa rugosa invaded coastal grassland communities in Belgium

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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-mass area (LMA)

Getting Started

Step 1. Installation

```
## Loading required package: usethis
```

```
##
## Attaching package: 'remotes'
```

```
## The following objects are masked from 'package:devtools':
##
##     dev_package_deps, install_bioc, install_bitbucket, install_cran,
##     install_deps, install_dev, install_git, install_github,
##     install_gitlab, install_local, install_svn, install_url,
##     install_version, update_packages
```

```
## The following object is masked from 'package:usethis':
##
##     git_credentials
```

```
##
## Attaching package: 'pls'
```

```
## The following object is masked from 'package:stats':
##
##     loadings
```

```
##
## 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/Data/GitHub/PLSR_for_plant_trait_prediction
```

```
##
## Attaching package: 'gridExtra'
```

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

Step 2. Setup other functions and options

```
### Setup other functions and options
github_dir <- file.path(here::here(),"R_Scripts")
source_from_gh <- TRUE
if (source_from_gh) {
  # Source helper functions from GitHub
  print("*** GitHub hash of functions.R file:")
  devtools::source_url("https://raw.githubusercontent.com/TESTgroup-BNL/PLSR_for_plant_trait_prediction/
} else {
  functions <- file.path(github_dir,"functions.R")
  source(functions)
}
```

```
## [1] "*** GitHub hash of functions.R file:"
```

```
## SHA-1 hash of file is 8c6b457e931a5879e8c975f4d6529f61b52c4453
```

```
# not in
`%notin%` <- Negate(`%in%`)

# 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 <- "LMA_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"
```

Step 3. Set working directory (scratch space)

```
## [1] "/private/var/folders/xp/h3k9vf3n2jx181ts786_yjrn9c2gjq/T/Rtmpm5uxwU"
```

Step 4. Pull example dataset from EcoSIS (ecosis.or)

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

URL: https://ecosis.org/package/fresh-leaf-spectra-to-estimate-lma-over-neon-domains-in-eastern-united-states

```
## [1] "Output directory: /Users/sserbin/Data/GitHub/PLSR_for_plant_trait_prediction/vignettes"
```

```
### Get source dataset from EcoSIS
dat_raw <- get_ecosis_data(ecosis_id = ecosis_id)
```

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

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

```
##
## -- Column specification --------------------------------------------------------
## cols(
##   .default = col_double(),
##   `Latin Species` = col_character(),
##   ids = col_character(),
##   `plot code` = col_character(),
##   `species code` = col_character()
## )
## i Use `spec()` for the full column specifications.
```

```
## Download complete!
```

```
head(dat_raw)
```

```
## # A tibble: 6 x 2,164
##   `Cw/EWT (cm3/cm~ `Latin Species` `Leaf area (mm2~ `Leaf calcium c~
##              <dbl> <chr>                      <dbl>            <dbl>
## 1          0.00887 Arrhenatherum ~             696.           0.0291
## 2          0.00824 Bromus sterilis             447.           0.0230
## 3          0.0280  Jacobaea vulga~            2418.           0.0950
## 4          0.0106  Rubus caesius              5719.           0.0700
## 5          0.00851 Arrhenatherum ~             671.           0.0286
## 6          0.0153  Crepis capilla~            1401.           0.0470
## # ... with 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>,
## #   `Plant height vegetative (cm)` <dbl>, ids <chr>, `plot code` <chr>,
## #   `species code` <chr>, `350` <dbl>, `351` <dbl>, `352` <dbl>, `353` <dbl>,
## #   `354` <dbl>, `355` <dbl>, `356` <dbl>, `357` <dbl>, `358` <dbl>,
## #   `359` <dbl>, `360` <dbl>, `361` <dbl>, `362` <dbl>, `363` <dbl>,
## #   `364` <dbl>, `365` <dbl>, `366` <dbl>, `367` <dbl>, `368` <dbl>,
## #   `369` <dbl>, `370` <dbl>, `371` <dbl>, `372` <dbl>, `373` <dbl>,
## #   `374` <dbl>, `375` <dbl>, `376` <dbl>, `377` <dbl>, `378` <dbl>,
## #   `379` <dbl>, `380` <dbl>, `381` <dbl>, `382` <dbl>, `383` <dbl>,
## #   `384` <dbl>, `385` <dbl>, `386` <dbl>, `387` <dbl>, `388` <dbl>,
## #   `389` <dbl>, `390` <dbl>, `391` <dbl>, `392` <dbl>, `393` <dbl>,
## #   `394` <dbl>, `395` <dbl>, `396` <dbl>, `397` <dbl>, `398` <dbl>,
## #   `399` <dbl>, `400` <dbl>, `401` <dbl>, `402` <dbl>, `403` <dbl>,
## #   `404` <dbl>, `405` <dbl>, `406` <dbl>, `407` <dbl>, `408` <dbl>,
```

```
## #   `409` <dbl>, `410` <dbl>, `411` <dbl>, `412` <dbl>, `413` <dbl>,
## #   `414` <dbl>, `415` <dbl>, `416` <dbl>, `417` <dbl>, `418` <dbl>,
## #   `419` <dbl>, `420` <dbl>, `421` <dbl>, `422` <dbl>, `423` <dbl>,
## #   `424` <dbl>, `425` <dbl>, `426` <dbl>, `427` <dbl>, `428` <dbl>,
## #   `429` <dbl>, `430` <dbl>, `431` <dbl>, `432` <dbl>, `433` <dbl>,
## #   `434` <dbl>, `435` <dbl>, `436` <dbl>, `437` <dbl>, `438` <dbl>,
## #   `439` <dbl>, `440` <dbl>, ...
```

```
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"
```

Step 5. 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/cm~ `Latin Species` `Leaf area (mm2~ `Leaf calcium c~
##              <dbl> <chr>                      <dbl>            <dbl>
## 1          0.00887 Arrhenatherum ~             696.           0.0291
## 2          0.00824 Bromus sterilis             447.           0.0230
## 3          0.0280  Jacobaea vulga~            2418.           0.0950
## 4          0.0106  Rubus caesius              5719.           0.0700
## 5          0.00851 Arrhenatherum ~             671.           0.0286
## 6          0.0153  Crepis capilla~            1401.           0.0470
## # ... with 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>,
## #   `species code` <chr>
```

```
sample_info2 <- sample_info %>%
  select(Plant_Species=`Latin Species`,Species_Code=`species code`,Plot=`plot code`,
         LMA_g_cm2=`Leaf mass per area (g/cm2)`)
sample_info2 <- sample_info2 %>%
  mutate(LMA_g_m2=LMA_g_cm2*10000)
head(sample_info2)
```

```
## # A tibble: 6 x 5
##   Plant_Species         Species_Code Plot  LMA_g_cm2 LMA_g_m2
##   <chr>                 <chr>        <chr>     <dbl>    <dbl>
## 1 Arrhenatherum elatius Arrela       DC1     0.00342     34.2
## 2 Bromus sterilis       Broste       DC1     0.00282     28.2
## 3 Jacobaea vulgaris     Jacvul       DC1     0.00417     41.7
## 4 Rubus caesius         Rubcae       DC1     0.00566     56.6
## 5 Arrhenatherum elatius Arrela       DC2     0.00361     36.1
## 6 Crepis capillaris     Creves       DC2     0.00283     28.3
```

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

Step 6. Example data cleaning.

```
plsr_data <- plsr_data[complete.cases(plsr_data[,names(plsr_data) %in%
                                                c(inVar,paste0("Wave_",wv))]),]
```

Step 7. Create cal/val datasets

```
method <- "dplyr" #base/dplyr
# base R - a bit slow
# dplyr - much faster
split_data <- create_data_split(approach=method, split_seed=7529075, 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  LMA_g_cm2 LMA_g_m2 Wave_500 Wave_501
## 1 Ammophila arenaria       Ammare  MC2 0.01679492 167.9492 0.135785  0.13685
## 2 Ammophila arenaria       Ammare  WC3 0.01844376 184.4376 0.151750  0.15275
## 3 Ammophila arenaria       Ammare  MC4 0.02030190 203.0190 0.156830  0.15790
## 4 Ammophila arenaria       Ammare  ZC2 0.01591894 159.1894 0.144450  0.14525
## 5 Ammophila arenaria       Ammare  ZC1 0.01483469 148.3469 0.147665  0.14910
## 6 Ammophila arenaria       Ammare  ZC3 0.01802409 180.2409 0.130885  0.13175
##   Wave_502
## 1 0.138150
## 2 0.154150
## 3 0.159065
## 4 0.146220
## 5 0.150330
## 6 0.132750
```

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

```
##          Plant_Species Species_Code Plot   LMA_g_cm2  LMA_g_m2   Wave_500
## 184  Jacobaea vulgaris       Jacvul  WC2 0.003551614  35.51614 0.06736887
## 185 Potentilla reptans       Potrep  WC2 0.005586320  55.86320 0.07125000
## 186      Rubus caesius       Rubcae  WC2 0.005803902  58.03902 0.05993560
## 187      Urtica dioica       Urtdio  WC2 0.005215705  52.15705 0.06508300
## 188 Ammophila arenaria       Ammare  WC3 0.018443757 184.43757 0.15175000
## 189  Jacobaea vulgaris       Jacvul  WC3 0.004980002  49.80002 0.06805547
##       Wave_501   Wave_502
## 184 0.06870667 0.07014220
## 185 0.07235000 0.07368350
## 186 0.06162000 0.06352233
## 187 0.06625000 0.06758350
## 188 0.15275000 0.15415000
## 189 0.06938000 0.07093553
```

```
rm(split_data)

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

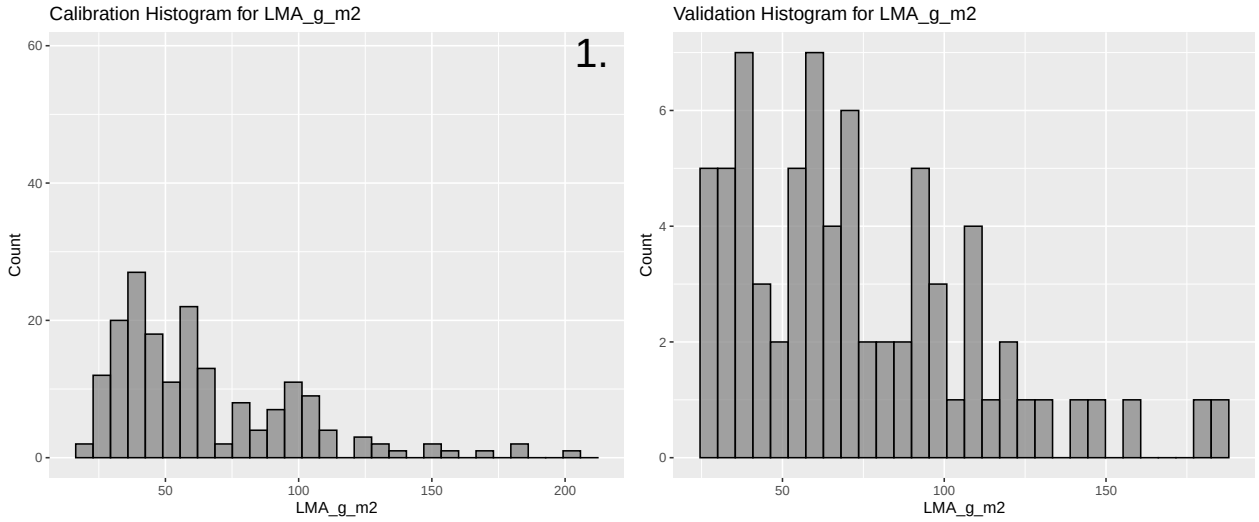
```
## [1] "Cal observations: 183"
```

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

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

```
text_loc <- c(max(hist(cal.plsr.data[,paste0(inVar)], plot=FALSE)$counts),
              max(hist(cal.plsr.data[,paste0(inVar)], plot=FALSE)$mids))
cal_hist_plot <- qplot(cal.plsr.data[,paste0(inVar)],geom="histogram",
                       main = paste0("Calibration Histogram for ",inVar),
                       xlab = paste0(inVar),ylab = "Count",fill=I("grey50"),col=I("black"),
                       alpha=I(.7)) +
  annotate("text", x=text_loc[2], y=text_loc[1], label= "1.",size=10)
val_hist_plot <- qplot(val.plsr.data[,paste0(inVar)],geom="histogram",
                       main = paste0("Validation Histogram for ",inVar),
                       xlab = paste0(inVar),ylab = "Count",fill=I("grey50"),col=I("black"),
                       alpha=I(.7))
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)
```

Step 8. 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  LMA_g_cm2 LMA_g_m2
## 1 Ammophila arenaria       Ammare  MC2 0.01679492 167.9492
## 2 Ammophila arenaria       Ammare  WC3 0.01844376 184.4376
## 3 Ammophila arenaria       Ammare  MC4 0.02030190 203.0190
```

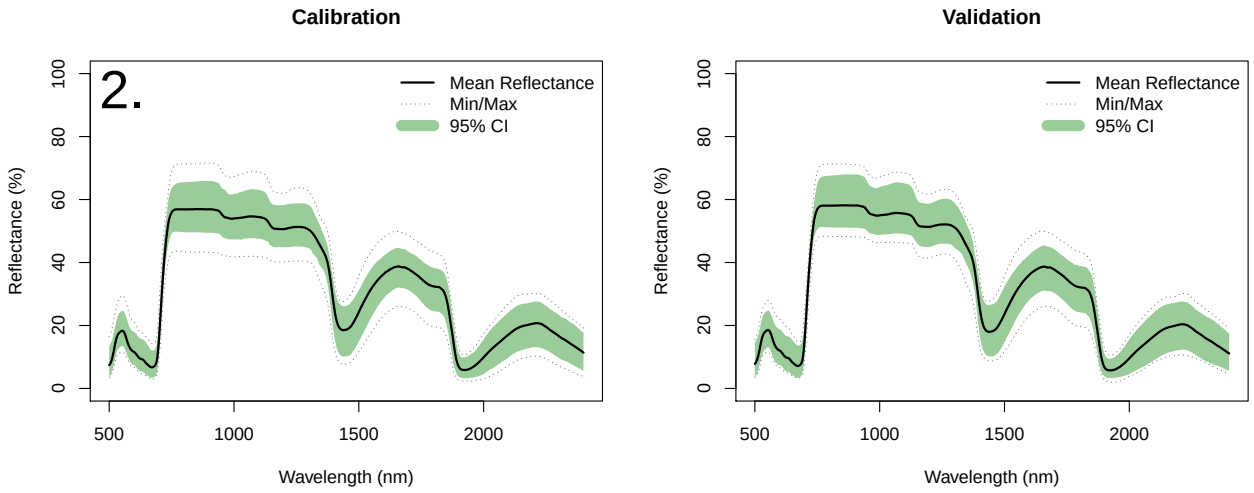
```
## 4 Ammophila arenaria       Ammare  ZC2 0.01591894 159.1894
## 5 Ammophila arenaria       Ammare  ZC1 0.01483469 148.3469
## 6 Ammophila arenaria       Ammare  ZC3 0.01802409 180.2409
```

```
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   LMA_g_cm2  LMA_g_m2
## 184  Jacobaea vulgaris       Jacvul  WC2 0.003551614  35.51614
## 185 Potentilla reptans       Potrep  WC2 0.005586320  55.86320
## 186      Rubus caesius       Rubcae  WC2 0.005803902  58.03902
## 187      Urtica dioica       Urtdio  WC2 0.005215705  52.15705
## 188 Ammophila arenaria       Ammare  WC3 0.018443757 184.43757
## 189  Jacobaea vulgaris       Jacvul  WC3 0.004980002  49.80002
```

Step 9. Calibration and Validation spectra plot

```
par(mfrow=c(1,2)) # B, L, T, R
f.plot.spec(Z=cal.plsr.data$Spectra,wv=seq(Start.wave,End.wave,1),
            plot_label="Calibration")
text(550,95,labels = "2.",cex=3)
f.plot.spec(Z=val.plsr.data$Spectra,wv=seq(Start.wave,End.wave,1),
            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
```

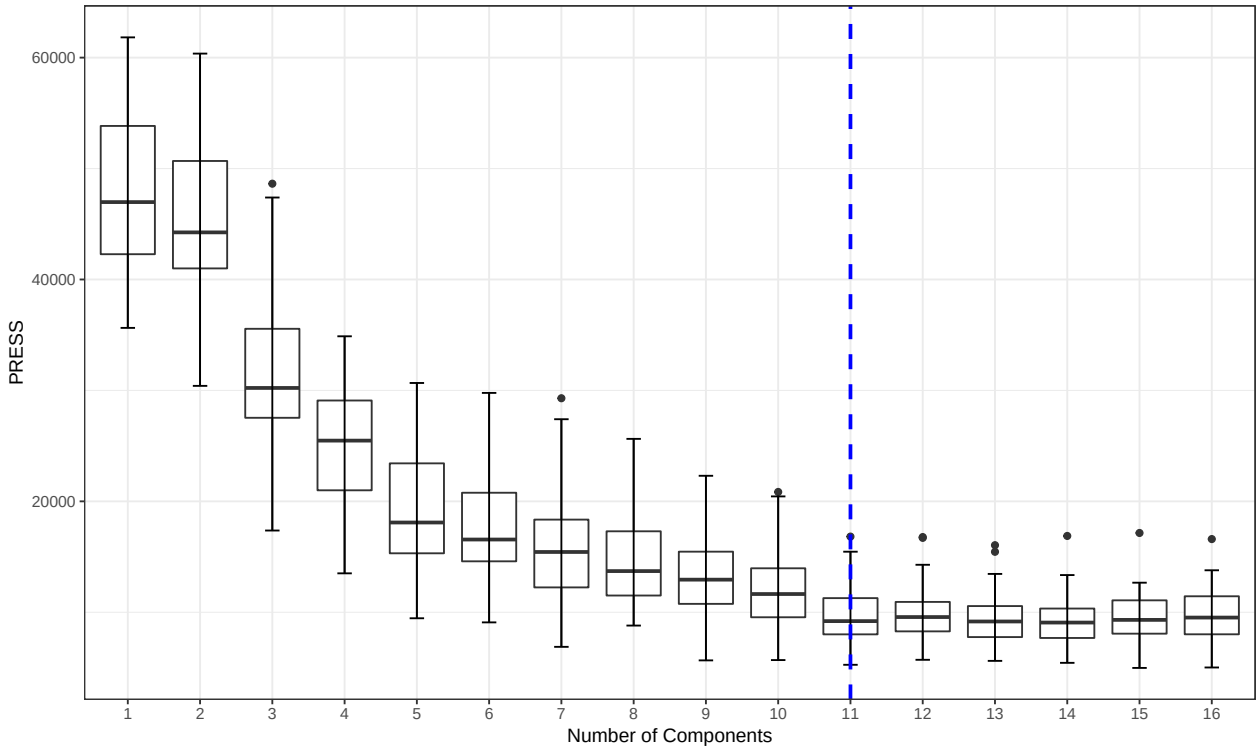
```
par(mfrow=c(1,1))
```

Step 10. Use permutation to determine the 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 <- "firstMin" #pls, firstPlateau, firstMin
random_seed <- 7529075
seg <- 80
maxComps <- 16
iterations <- 50
prop <- 0.70
if (method=="pls") {
  nComps <- find_optimal_components(dataset=cal.plsr.data, method=method, maxComps=maxComps,
                                    seg=seg, random_seed=random_seed)
  print(paste0("*** Optimal number of components: ", nComps))
} else {
  nComps <- find_optimal_components(dataset=cal.plsr.data, method=method, maxComps=maxComps,
                                    iterations=iterations, seg=seg, prop=prop,
                                    random_seed=random_seed)
}
```

```
## [1] "*** Running permutation test.  Please hang tight, this can take awhile ***"
## [1] "Options: 16 50 80 0.7"
## Running interation 1
## Running interation 2
## Running interation 3
## Running interation 4
## Running interation 5
## Running interation 6
## Running interation 7
## Running interation 8
## Running interation 9
## Running interation 10
## Running interation 11
## Running interation 12
## Running interation 13
## Running interation 14
## Running interation 15
## Running interation 16
## Running interation 17
## Running interation 18
```

```
## Running interation 19
## Running interation 20
## Running interation 21
## Running interation 22
## Running interation 23
## Running interation 24
## Running interation 25
## Running interation 26
## Running interation 27
## Running interation 28
## Running interation 29
## Running interation 30
## Running interation 31
## Running interation 32
## Running interation 33
## Running interation 34
## Running interation 35
## Running interation 36
## Running interation 37
## Running interation 38
## Running interation 39
## Running interation 40
## Running interation 41
## Running interation 42
## Running interation 43
## Running interation 44
## Running interation 45
## Running interation 46
## Running interation 47
## Running interation 48
## Running interation 49
## Running interation 50
## No id variables; using all as measure variables
## [1] "*** Optimal number of components based on t.test: 11"
```




```
print("*** Figure 3. Optimal PLSR component selection ***")
```

```
## [1] "*** Figure 3. Optimal PLSR component selection ***"
```

```
dev.copy(png,file.path(outdir,paste0(paste0("Figure_3_",inVar,
                                            "_PLSR_Component_Selection.png"))),
         height=2800, width=3400,  res=340)
```

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

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

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

Step 11. Fit final model

```
### Fit final model - using leave-one-out cross validation
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
text_loc <- c(max(RMSEP(plsr.out, newdata = val.plsr.data)$comps),
              RMSEP(plsr.out, newdata = val.plsr.data)$val[1])
par(mfrow=c(1,2)) # B, L, T, R
RMSEP(plsr.out, newdata = val.plsr.data)
```

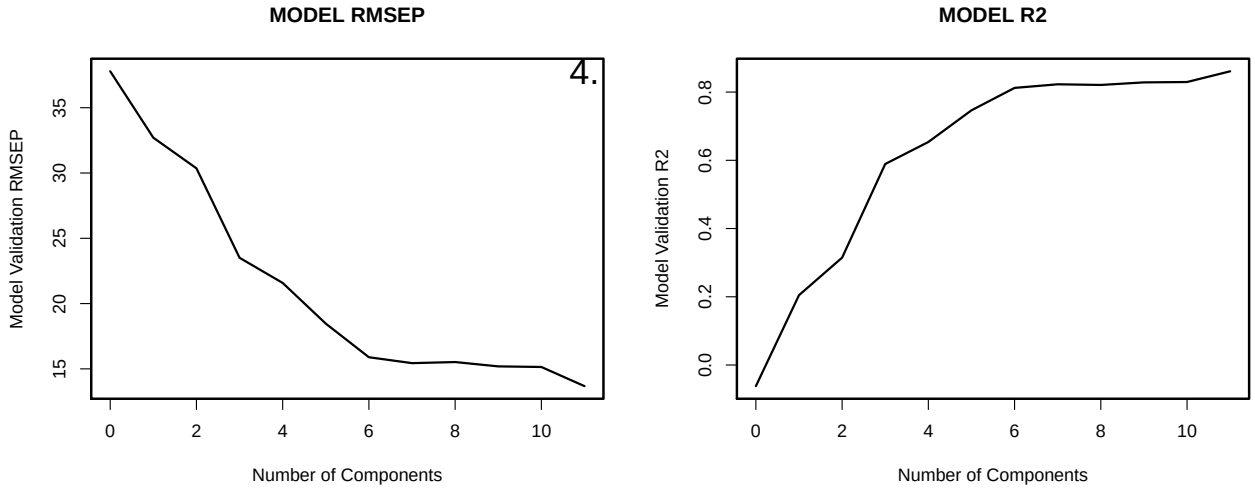
```
## (Intercept)      1 comps      2 comps      3 comps      4 comps      5 comps
```

```
##       37.79        32.71        30.36        23.51        21.58        18.46
##     6 comps      7 comps      8 comps      9 comps     10 comps     11 comps
##       15.89        15.44        15.52        15.19        15.14        13.68
```

```
plot(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)
text(text_loc[1],text_loc[2],labels = "4.", cex=2)
box(lwd=2.2)

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

```
## (Intercept)      1 comps      2 comps      3 comps      4 comps      5 comps
##    -0.06195      0.20461      0.31467      0.58911      0.65365      0.74649
##     6 comps      7 comps      8 comps      9 comps     10 comps     11 comps
##     0.81222      0.82276      0.82084      0.82841      0.82945      0.86090
```

```
plot(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)
```

Step 12. 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  LMA_g_cm2 LMA_g_m2 PLSR_Predicted
## 1 Ammophila arenaria       Ammare  MC2 0.01679492 167.9492       154.1892
## 2 Ammophila arenaria       Ammare  WC3 0.01844376 184.4376       147.0878
## 3 Ammophila arenaria       Ammare  MC4 0.02030190 203.0190       153.8674
## 4 Ammophila arenaria       Ammare  ZC2 0.01591894 159.1894       161.6047
## 5 Ammophila arenaria       Ammare  ZC1 0.01483469 148.3469       144.9268
## 6 Ammophila arenaria       Ammare  ZC3 0.01802409 180.2409       148.2100
##   PLSR_CV_Predicted PLSR_CV_Residuals
## 1          151.7161        -16.233027
## 2          137.3863        -47.051273
## 3          144.2584        -58.760574
## 4          162.6250          3.435614
## 5          142.9101         -5.436767
## 6          142.5160        -37.724928
```

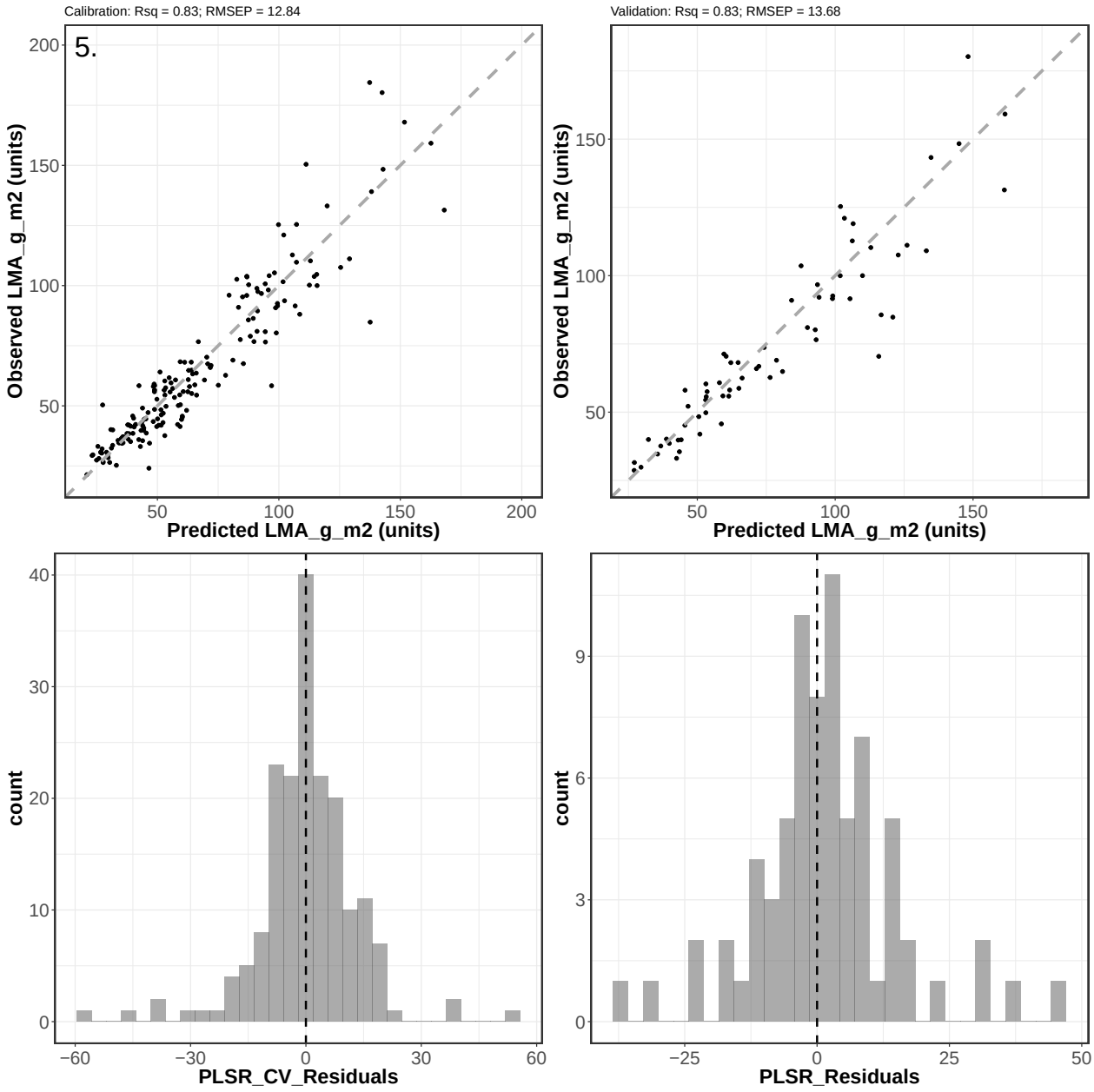
```
cal.R2 <- round(pls::R2(plsr.out)[[1]][nComps],2)
cal.RMSEP <- round(sqrt(mean(cal.plsr.output$PLSR_CV_Residuals^2)),2)

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   LMA_g_cm2  LMA_g_m2 PLSR_Predicted
## 1  Jacobaea vulgaris       Jacvul  WC2 0.003551614  35.51614       43.51586
## 2 Potentilla reptans       Potrep  WC2 0.005586320  55.86320       61.41726
## 3      Rubus caesius       Rubcae  WC2 0.005803902  58.03902       45.55789
## 4      Urtica dioica       Urtdio  WC2 0.005215705  52.15705       46.65139
## 5 Ammophila arenaria       Ammare  WC3 0.018443757 184.43757      147.08781
## 6  Jacobaea vulgaris       Jacvul  WC3 0.004980002  49.80002       53.09532
##   PLSR_Residuals
## 1       7.999719
## 2       5.554059
## 3     -12.481126
## 4      -5.505664
## 5     -37.349758
## 6       3.295298
```

```
val.R2 <- round(pls::R2(plsr.out,newdata=val.plsr.data)[[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", size=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, size=1.5)) +
  annotate("text", x=rng_quant[1], y=rng_quant[2], label= "5.",size=10)

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", size=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, size=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", size=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, size=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", size=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, size=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 6 rows containing missing values (geom_point).

## Warning: Removed 6 rows containing missing values (geom_point).

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

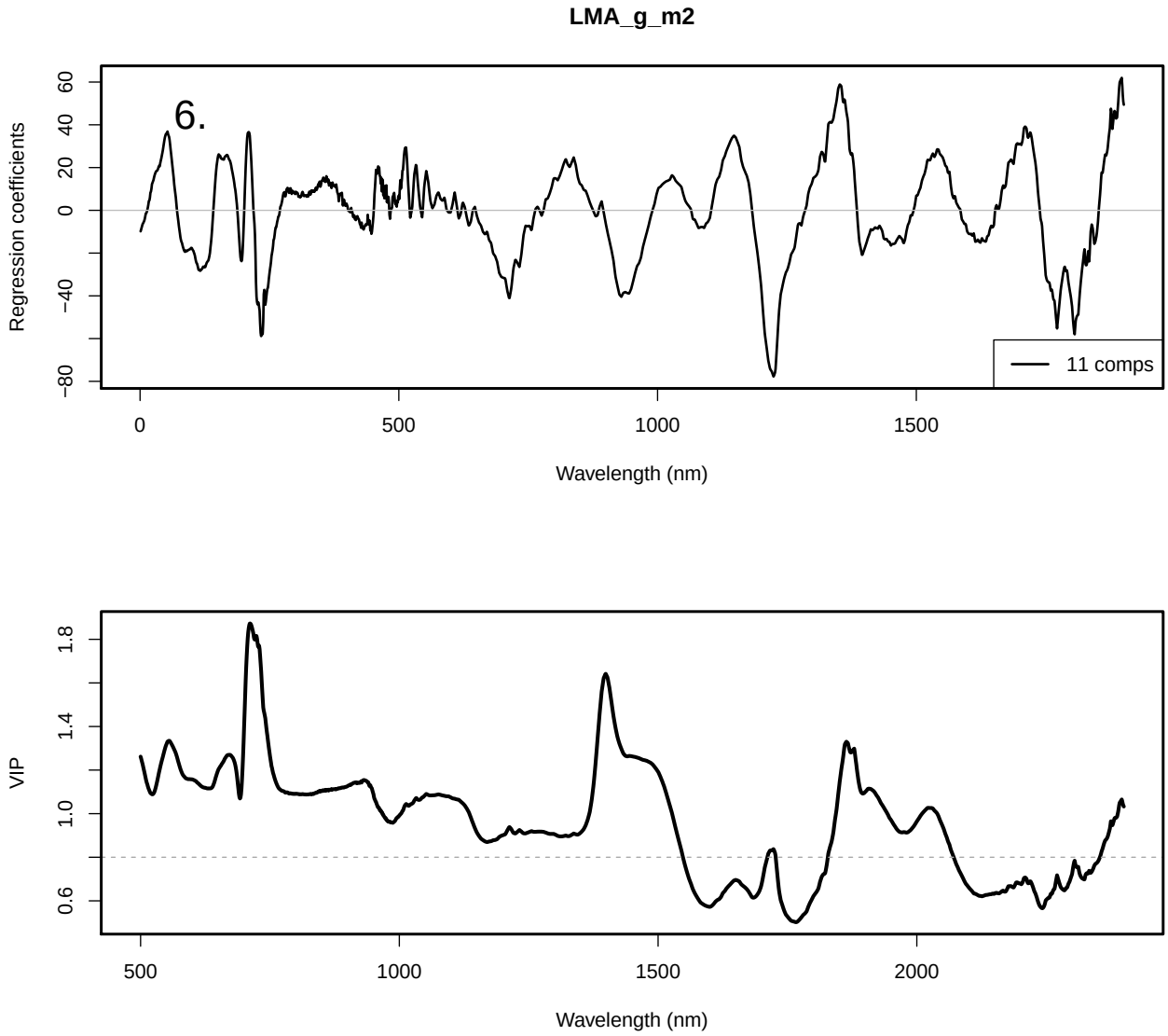



```
ggsave(filename = file.path(outdir,paste0(inVar,"_Cal_Val_Scatterplots.png")),
       plot = scatterplots, device="png", width = 32, height = 30, units = "cm",
       dpi = 300)
```

Step 13. Generate Coefficient and VIP plots

```
vips <- 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)
legend("topleft",legend = "6.", cex=2, bty="n")
```

```
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
```

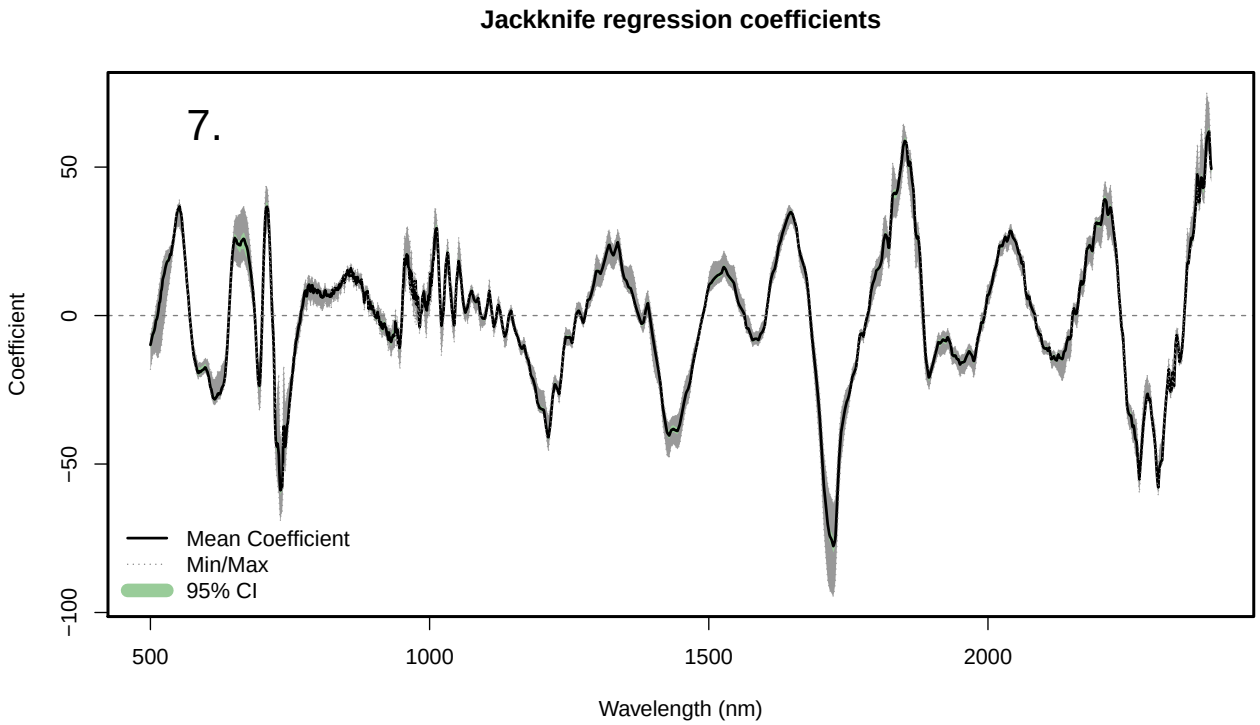
Step 14. Permutation analysis to derive uncertainty estimates

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

jk.plsr.out <- pls::plsr(as.formula(paste(inVar,"~","Spectra")), scale=FALSE,
                         center=TRUE, ncomp=nComps, validation="LOO", trace=FALSE,
                         jackknife=TRUE,
                         data=cal.plsr.data)
pls.options(parallel = NULL)

Jackknife_coef <- f.coef.valid(plsr.out = jk.plsr.out, data_plsr = cal.plsr.data,
                               ncomp = nComps, inVar=inVar)
Jackknife_intercept <- Jackknife_coef[1,,,]
Jackknife_coef <- Jackknife_coef[2:dim(Jackknife_coef)[1],,,]

interval <- c(0.025,0.975)
Jackknife_Pred <- val.plsr.data$Spectra %*% Jackknife_coef +
  matrix(rep(Jackknife_intercept, length(val.plsr.data[,inVar])), byrow=TRUE,
         ncol=length(Jackknife_intercept))
Interval_Conf <- apply(X = Jackknife_Pred, MARGIN = 1, FUN = quantile,
                       probs=c(interval[1], interval[2]))
sd_mean <- apply(X = Jackknife_Pred, MARGIN = 1, FUN =sd)
sd_res <- sd(val.plsr.output$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   LMA_g_cm2  LMA_g_m2 PLSR_Predicted
## 1  Jacobaea vulgaris       Jacvul  WC2 0.003551614  35.51614       43.51586
## 2 Potentilla reptans       Potrep  WC2 0.005586320  55.86320       61.41726
## 3      Rubus caesius       Rubcae  WC2 0.005803902  58.03902       45.55789
## 4      Urtica dioica       Urtdio  WC2 0.005215705  52.15705       46.65139
## 5 Ammophila arenaria       Ammare  WC3 0.018443757 184.43757      147.08781
## 6  Jacobaea vulgaris       Jacvul  WC3 0.004980002  49.80002       53.09532
##   PLSR_Residuals       LCI       UCI       LPI       UPI
## 1       7.999719  42.58086  44.15724  16.70642  70.32530
## 2       5.554059  60.10507  62.52674  34.59536  88.23916
## 3     -12.481126  44.66849  48.22967  18.70489  72.41090
## 4      -5.505664  45.70375  47.84938  19.82512  73.47765
## 5     -37.349758 145.09309 148.61694 120.18052 173.99510
## 6       3.295298  52.40880  53.97806  26.28498  79.90565
```

```
### Permutation coefficient plot
f.plot.coef(Z = t(Jackknife_coef), wv = seq(Start.wave,End.wave,1),
            plot_label="Jackknife regression coefficients",position = 'bottomleft')
abline(h=0,lty=2,col="grey50")
legend("topleft",legend = "7.", cex=2, bty="n")
```

```
box(lwd=2.2)
```




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

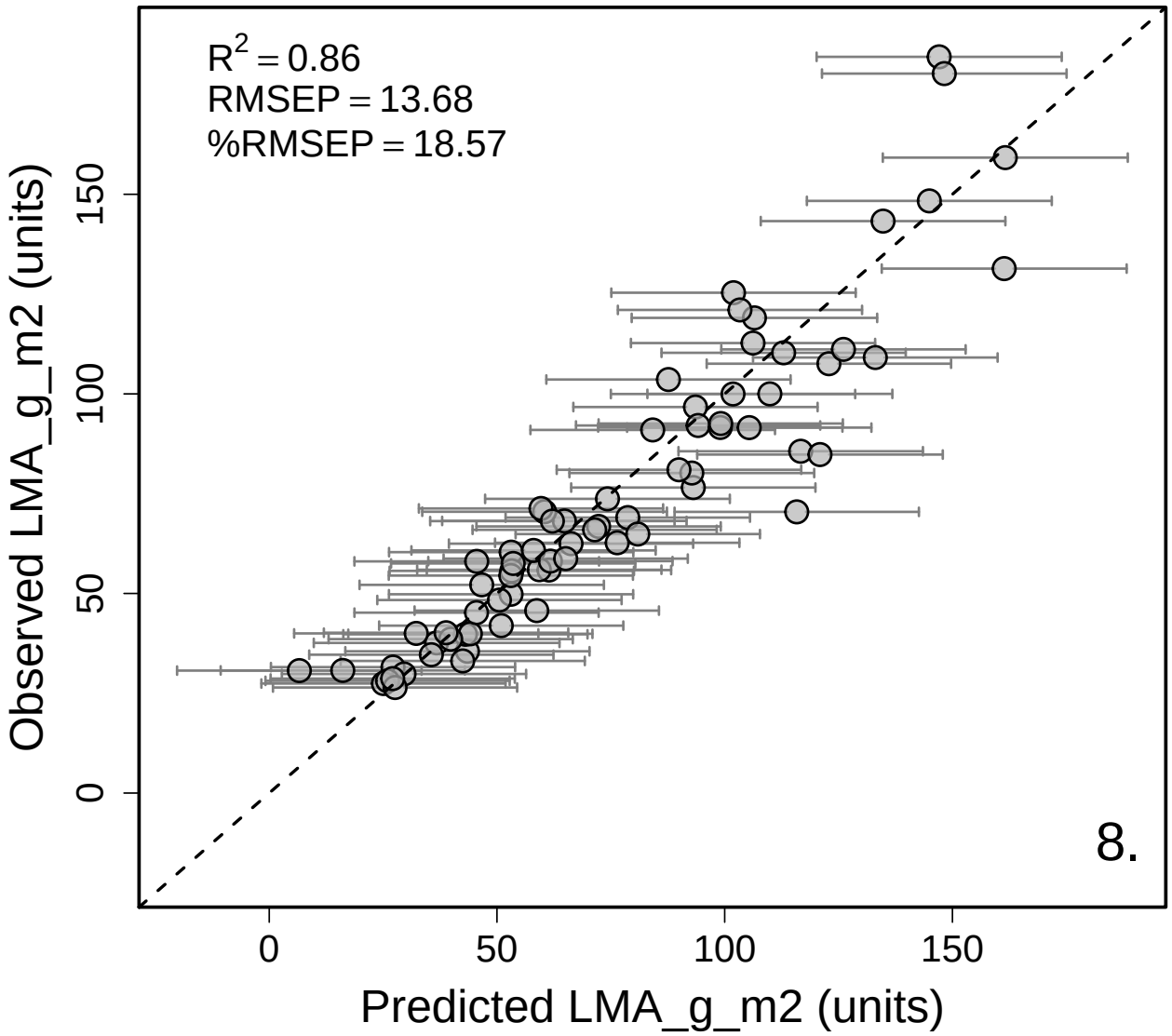
```
## quartz_off_screen
##                 3
```

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

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

```
### Permutation validation plot
RMSEP <- sqrt(mean(val.plsr.output$PLSR_Residuals^2))
pecr_RMSEP <- RMSEP/mean(val.plsr.output[,inVar])*100
r2 <- round(pls::R2(plsr.out, newdata = val.plsr.data)$val[nComps+1],2)
expr <- vector("expression", 3)
expr[[1]] <- bquote(R^2==.(r2))
expr[[2]] <- bquote(RMSEP==.(round(RMSEP,2)))
expr[[3]] <- bquote("%RMSEP"==.(round(pecr_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))
plotCI(val.plsr.output$PLSR_Predicted,val.plsr.output[,inVar],
       li=val.plsr.output$LPI, ui=val.plsr.output$UPI, gap=0.009,sfrac=0.004,
       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=alpha("grey70",0.7), scol="grey50",
       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)
legend("topleft", legend=expr, bty="n", cex=1.5)
```

```
legend("bottomright", legend="8.", bty="n", cex=2.2)
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
```

Step 15. Output permutation coefficients for later use

```
out.jk.coefs <- data.frame(Iteration=seq(1,length(Jackknife_intercept),1),
                           Intercept=Jackknife_intercept,t(Jackknife_coef))
```

```r
head(out.jk.coefs)[1:6]
```

```
##       Iteration Intercept   Wave_500   Wave_501   Wave_502   Wave_503
## Seg 1         1  18.33909  -7.580446  -6.724083  -5.886226  -4.984744
## Seg 2         2  21.22164  -8.574931  -7.084795  -6.255716  -5.384000
## Seg 3         3  19.63843 -18.104491 -17.260522 -16.154983 -14.960119
## Seg 4         4  15.90905 -10.715594  -9.874766  -8.926979  -8.007834
## Seg 5         5  17.51805  -8.952143  -8.305344  -7.136167  -6.221407
## Seg 6         6  12.18563  -7.702160  -7.128890  -6.532276  -5.840220
```

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

Step 16. Output remaining core PLSR outputs

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

```
## [1] "Output directory:  /var/folders/xp/h3k9vf3n2jx181ts786_yjrn9c2gjq/T//Rtmpm5uxwU"
```

```r
# 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')))
```

Step 17. Confirm files were written to temp space

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

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

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

```
##  [1] "Figure_3_LMA_g_m2_PLSR_Component_Selection.png"
```

```
##  [2] "LMA_g_m2_Cal_PLSR_Dataset.csv"
##  [3] "LMA_g_m2_Cal_Val_Histograms.png"
##  [4] "LMA_g_m2_Cal_Val_Scatterplots.png"
##  [5] "LMA_g_m2_Cal_Val_Spectra.png"
##  [6] "LMA_g_m2_Coefficient_VIP_plot.png"
##  [7] "LMA_g_m2_Jackkife_PLSR_Coefficients.csv"
##  [8] "LMA_g_m2_Jackknife_Regression_Coefficients.png"
##  [9] "LMA_g_m2_Observed_PLSR_CV_Pred_11comp.csv"
## [10] "LMA_g_m2_PLSR_Coefficients_11comp.csv"
## [11] "LMA_g_m2_PLSR_Validation_Scatterplot.png"
## [12] "LMA_g_m2_PLSR_VIPs_11comp.csv"
## [13] "LMA_g_m2_Val_PLSR_Dataset.csv"
## [14] "LMA_g_m2_Validation_PLSR_Pred_11comp.csv"
## [15] "LMA_g_m2_Validation_RMSEP_R2_by_Component.png"
```