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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2022-03-15

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. Load libraries needed to run example script

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

```
##
## 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/spectratrait

##
## Attaching package: 'gridExtra'

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

Step 2. 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 <- "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/RtmpbOFk4k"
```

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

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

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

```
### Get source dataset from EcoSIS
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!
```

```
head(dat_raw)
```

```
## # A tibble: 6 x 2,164
##   `Cw/EWT (cm3/cm2)` `Latin Species`       `Leaf area (mm2)` `Leaf calcium con~`
##                <dbl> <chr>                             <dbl>               <dbl>
## 1            0.00887 Arrhenatherum elatius              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 elatius              671.              0.0286
## 6            0.0153  Crepis capillaris                 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>, ...
```

```
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/cm2)` `Latin Species`       `Leaf area (mm2)` `Leaf calcium con~`
##                <dbl> <chr>                             <dbl>               <dbl>
## 1            0.00887 Arrhenatherum elatius              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 elatius              671.              0.0286
## 6            0.0153  Crepis capillaris                 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>, ...
```

```
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.

```
#### Example data cleaning.  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))]),]
```

Step 7. Create cal/val datasets

```
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=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
## 1   Arrhenatherum elatius       Arrela  DC1 0.003420518  34.20518 0.070667
## 2         Bromus sterilis       Broste  DC1 0.002816940  28.16940 0.105300
## 5   Arrhenatherum elatius       Arrela  DC2 0.003611619  36.11619 0.076300
## 6      Crepis capillaris       Creves  DC2 0.002828699  28.28699 0.062717
## 11         Carex arenaria       Carare  DC3 0.010579908 105.79908 0.115885
## 16       Elytrigia juncea       Elyjun  DC4 0.012400353 124.00353 0.116320
##    Wave_501 Wave_502
## 1   0.07160 0.072533
## 2   0.10710 0.109030
## 5   0.07670 0.077300
## 6   0.06365 0.064850
## 11  0.11705 0.118450
## 16  0.11745 0.118850
```

```
rm(split_data)

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

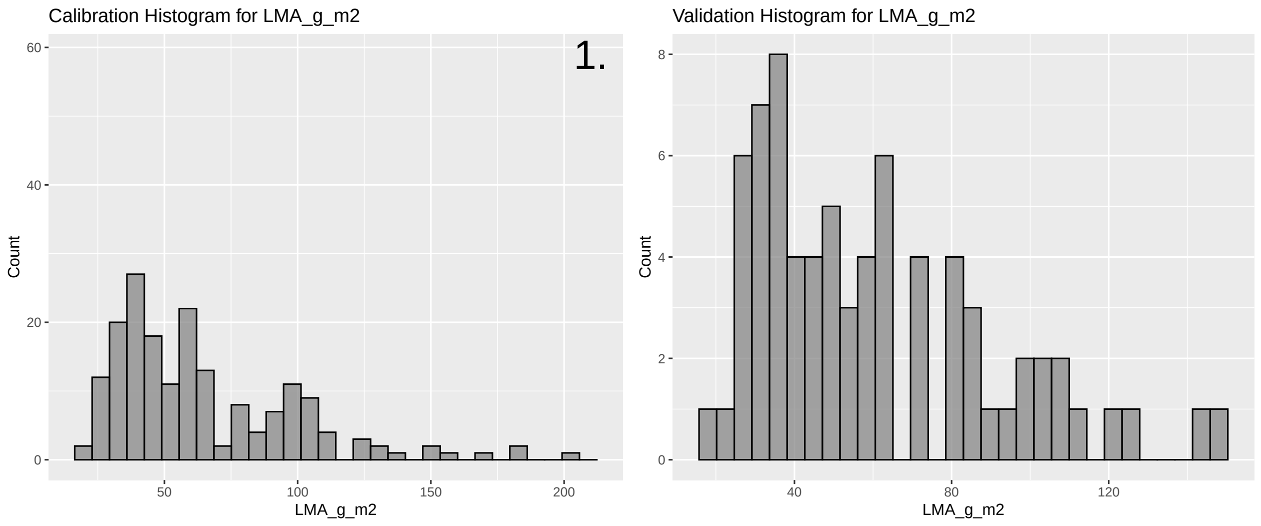
```
## [1] "Cal observations: 183"
```

```
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`.
```



```
# Figure S1. The resulting leaf mass area (LMA, g/m2) distribution (histogram) for the
# calibration (i.e. model training) and validation datasets. The data was split using
# the spectratrait::create_data_split() function using "Species_Code" as the
# group_variable and using a data split proportion per group of 80% to calibration
# and 20% to validation
```

```
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]
```

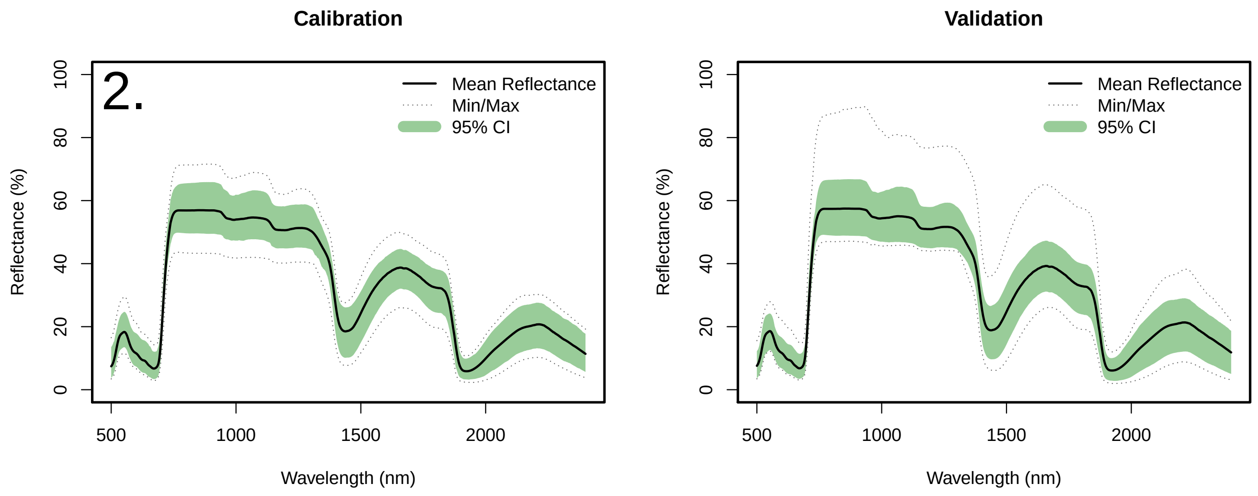
```
##         Plant_Species Species_Code Plot  LMA_g_cm2 LMA_g_m2
## 1 Ammophila arenaria       Ammare  MC2 0.01679492 167.9492
## 2 Ammophila arenaria       Ammare  WC3 0.01844376 184.4376
## 3 Ammophila arenaria       Ammare  MC4 0.02030190 203.0190
## 4 Ammophila arenaria       Ammare  ZC2 0.01591894 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
## 1  Arrhenatherum elatius       Arrela  DC1 0.003420518  34.20518
## 2        Bromus sterilis       Broste  DC1 0.002816940  28.16940
## 5  Arrhenatherum elatius       Arrela  DC2 0.003611619  36.11619
## 6      Crepis capillaris       Creves  DC2 0.002828699  28.28699
## 11        Carex arenaria       Carare  DC3 0.010579908 105.79908
## 16      Elytrigia juncea       Elyjun  DC4 0.012400353 124.00353
```

Step 9. Calibration and Validation spectra plot

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



```
# Figure S2. The resulting calibration and validation spectral reflectance distribution by
# wavelength. The spectra split was done at the same time as LMA, as described in
# Supplemental Figure S1.

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))
```

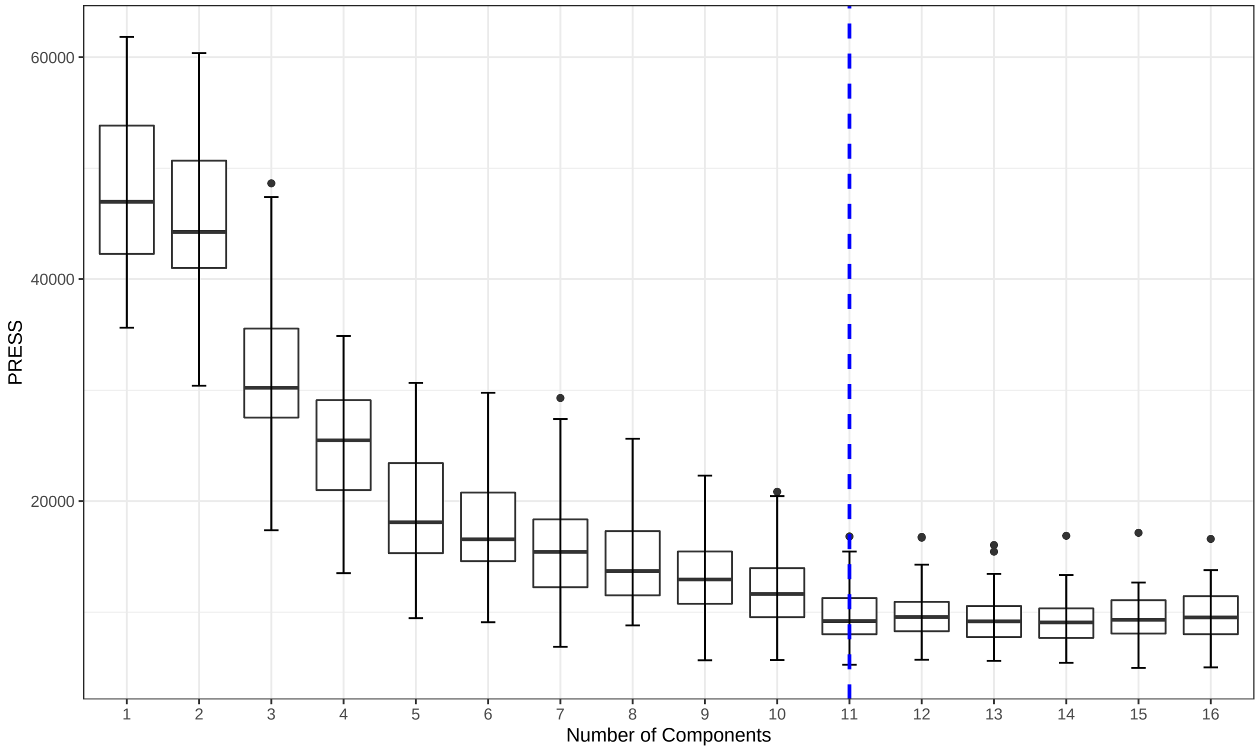
Step 10. Use permutation to determine the optimal number of components

```
### 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 <- 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 permutation test.  Please hang tight, this can take awhile ***"
## [1] "Options:"
## [1] "Max Components: 16 Iterations: 50 Data Proportion (percent): 70"
## [1] "*** Providing PRESS and coefficient array output ***"
```

```
## No id variables; using all as measure variables
```

```
## [1] "*** Optimal number of components based on t.test: 11"
```



```
# Figure S3. Selection of the optimal number of components based on the
# minimization of the PRESS statistic.  In this example we show "firstMin"
# option that selects the number of components corresponding to the first
# statistical minimum PRESS value (vertical broken blue line).

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

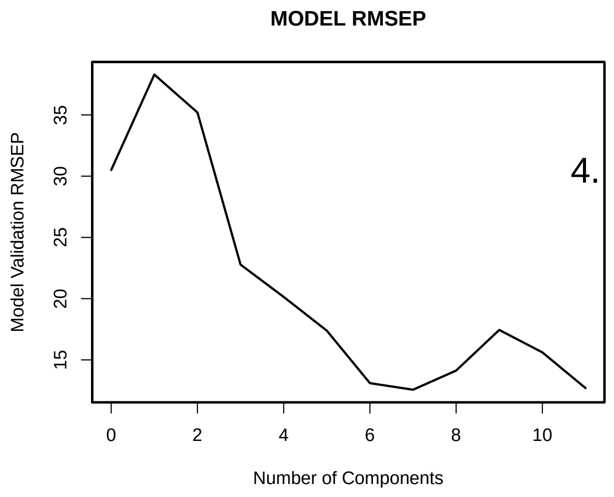
```
### Fit final model - using leave-one-out cross validation
plsr.out <- plsr(as.formula(paste(inVar,"~","Spectra")),scale=FALSE,ncomp=nComps,
                 validation="LOO",trace=FALSE,data=cal.plsr.data)
fit <- plsr.out$fitted.values[,1,nComps]
pls.options(parallel = NULL)

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

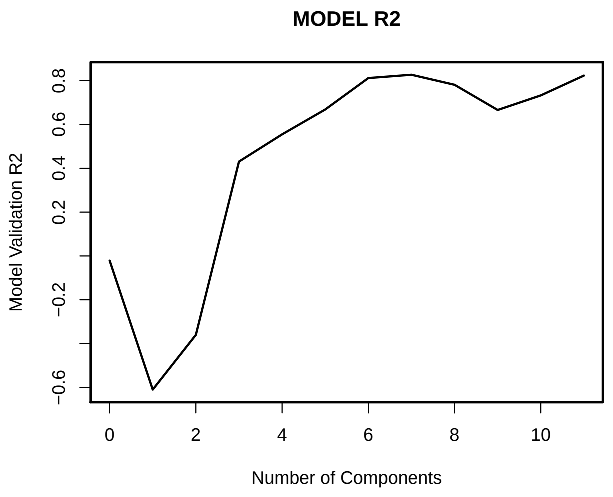
```
## (Intercept)      1 comps      2 comps      3 comps      4 comps      5 comps
##       30.50        38.30        35.20        22.78        20.14        17.39
##     6 comps      7 comps      8 comps      9 comps     10 comps     11 comps
##       13.10        12.56        14.13        17.45        15.61        12.70
```

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

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

```
## (Intercept)      1 comps      2 comps      3 comps      4 comps      5 comps
##    -0.02137     -0.60981     -0.36001      0.43050      0.55467      0.66818
##     6 comps      7 comps      8 comps      9 comps     10 comps     11 comps
##     0.81156      0.82673      0.78088      0.66593      0.73244      0.82292
```

```
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)
```





```
# Figure S4. A plot of the validation root mean square error of prediction (RMSEP, left)
# and coefficient of determination (right) for the 0 to optimal number of components

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,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)
```

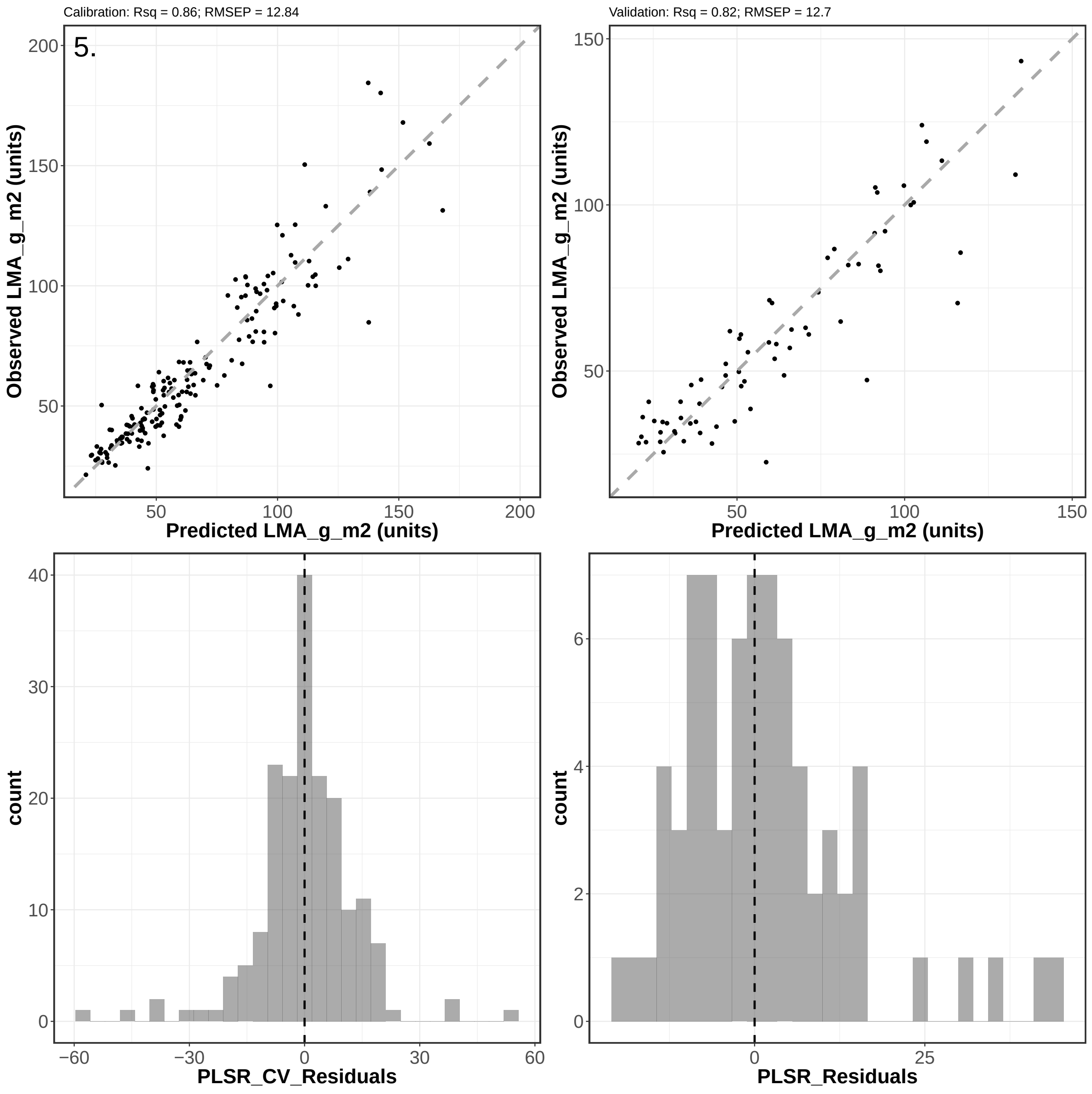
```
##            Plant_Species Species_Code Plot   LMA_g_cm2  LMA_g_m2 PLSR_Predicted
## 1  Arrhenatherum elatius       Arrela  DC1 0.003420518  34.20518       36.09345
## 2        Bromus sterilis       Broste  DC1 0.002816940  28.16940       42.52977
## 5  Arrhenatherum elatius       Arrela  DC2 0.003611619  36.11619       21.87053
## 6      Crepis capillaris       Creves  DC2 0.002828699  28.28699       20.66219
## 11         Carex arenaria      Carare  DC3 0.010579908 105.79908       99.79501
## 16      Elytrigia juncea       Elyjun  DC4 0.012400353 124.00353      105.16400
##    PLSR_Residuals
## 1        1.888268
## 2       14.360370
## 5      -14.245663
## 6       -7.624796
## 11      -6.004066
## 16     -18.839527
```

```
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", 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 3 rows containing missing values (geom_point).
```

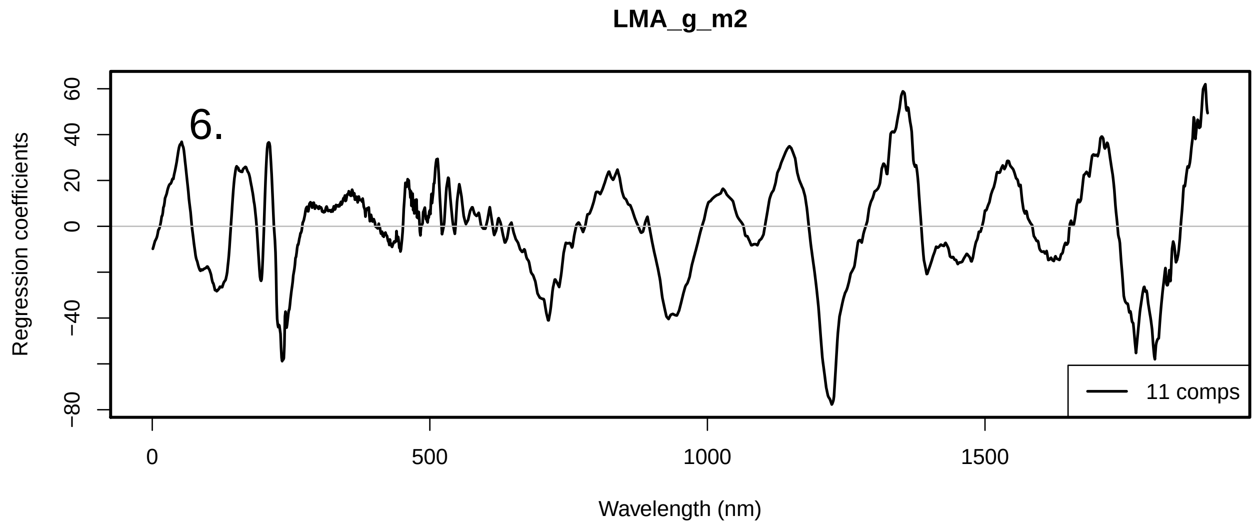
```
## `stat_bin()` using `bins = 30`. Pick better value with `binwidth`.
## `stat_bin()` using `bins = 30`. Pick better value with `binwidth`.
```

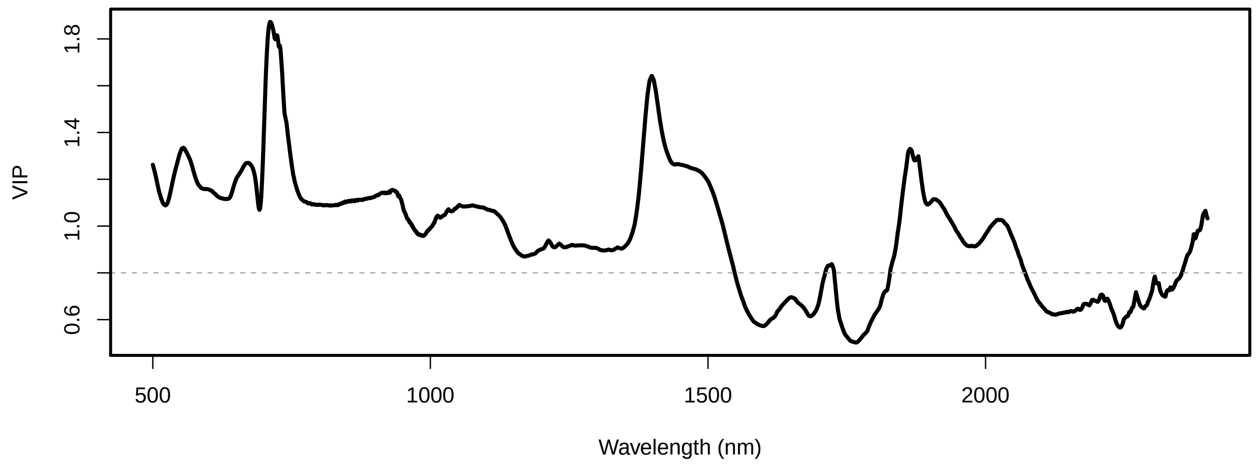


```
# Figure S5. The calibration model and independent validation scatter plot results for
# the example LMA PLSR model (top row). Also shown are the calibration model and
# validation PLSR residuals, where the calibration results are based on the internal
# model cross-validation and the validation residuals are the predicted minus observed
# values of LMA.
```

Step 13. 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)
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)
```





```
# Figure S6. The calibration model PLSR regression coefficient (top) and variable
# importance of projection (bottom) plots

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

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

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

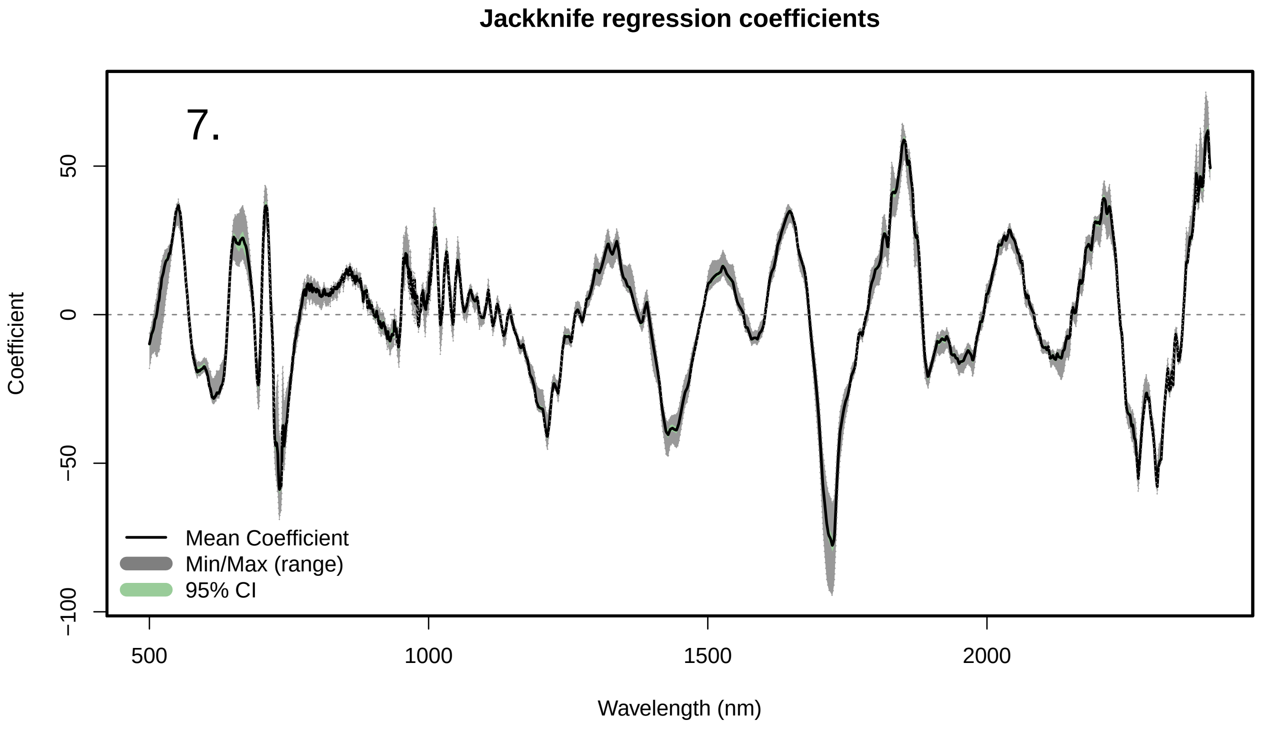
```
## pdf
##   2
```

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 <- spectratrait::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  Arrhenatherum elatius       Arrela  DC1 0.003420518  34.20518       36.09345
## 2        Bromus sterilis       Broste  DC1 0.002816940  28.16940       42.52977
## 5  Arrhenatherum elatius       Arrela  DC2 0.003611619  36.11619       21.87053
## 6      Crepis capillaris       Creves  DC2 0.002828699  28.28699       20.66219
## 11        Carex arenaria       Carare  DC3 0.010579908 105.79908       99.79501
## 16      Elytrigia juncea       Elyjun  DC4 0.012400353 124.00353      105.16400
##    PLSR_Residuals       LCI       UCI       LPI       UPI
## 1        1.888268  35.22975  36.83681 11.182998  61.00390
## 2       14.360370  41.61622  43.52851 17.617164  67.44238
## 5      -14.245663  20.07042  23.96996 -3.085793  46.82685
## 6       -7.624796  20.27384  21.15353 -4.234964  45.55935
## 11      -6.004066  98.52166 100.58017 74.888636 124.70139
## 16     -18.839527 104.18470 105.69273 80.260059 130.06795
```

```r
### Permutation coefficient plot
spectratrait::f.plot.coef(Z = t(Jackknife_coef), wv = wv,
              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)
```



```r
# Figure S7. The calibration model jackknife PLSR regression coefficients

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

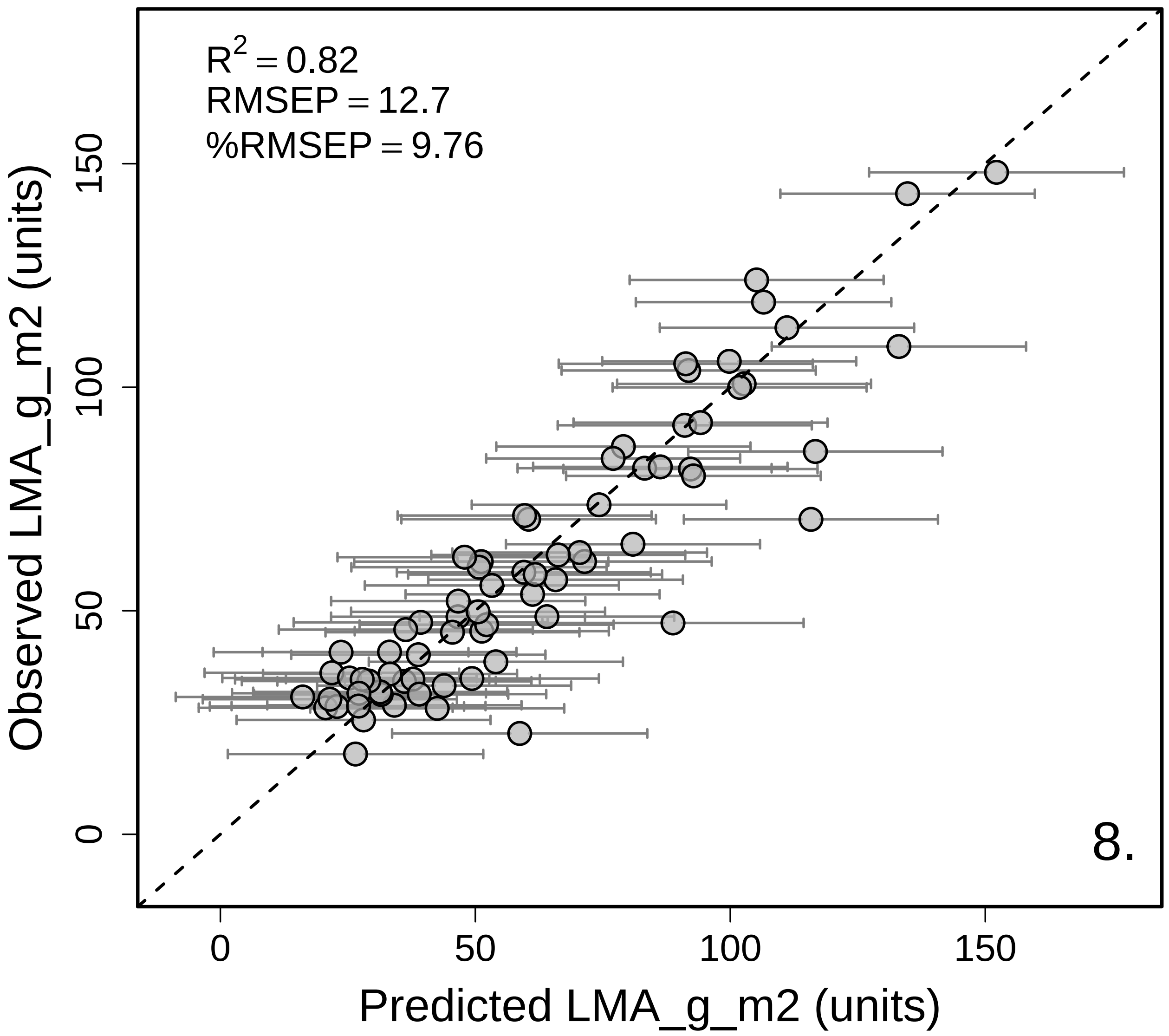
```
## quartz_off_screen
##                 3
```

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

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

```r
### Permutation 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)))
```

```r
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=0.004,
        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="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)
```



```r
# Figure S8. Independent validation results for the LMA PLSR model with associated
# jackknife uncertainty estimate 95% prediction intervals for each estimate LMA
# value. The %RMSEP is the model prediction performance standardized to the
# percentage of the response range, in this case the range of LMA values
```

```
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))
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
```

```
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

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

```
## [1] "Output directory:  /var/folders/xp/h3k9vf3n2jx181ts786_yjrn9c2gjq/T//Rtmpb0Fk4k"
```

```
# 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

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

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

```
print(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"
```