# Introduction

In recent years, the scale of data available for ecological research has increased due to advancements in high-throughput sampling technology (Kallenbach et al. 2014; Kfoury et al. 2017), image processing (Berger et al. 2010; Fahlgren et al. 2015), automated and remote data logging (Cooke et al. 2004; Porter et al. 2005), remote sensing (Roughgarden et al. 1991; Aplin 2005), high-throughput sequencing technologies (Soininen et al. 2009), and citizen science (Silvertown 2009; Bonney et al. 2009; Dickinson et al. 2012). Resulting large, multivariate datasets have the potential to increase our understanding of ecological phenomena, given that adequate statistical techniques are used to separate signal from potentially increasing noise.

Multivariate data is of course not a new phenomenon in ecological research. [something about community ecology/ species richness/ vegan package].

[I’m wondering if we should focus on regression approaches over discriminant approaches, only mentioning PLSDA at the end. This makes for less compelling figures, but more compelling novelty (like, we already have PERMANOVA for discriminant multivariate analysis, and in some ways it might be better than PLS-DA). The benefit of PLS-DA is, I guess, variable selection without post-hoc testing.]

Multivariate data can be analyzed in two fundamentally different ways—unsupervised, and supervised analyses. Unsupervised analyses describe patterns in the data and are often used in more descriptive studies. For example principal component analysis is a widely used unsupervised technique that describes the covariation among variables in as few axes as possible. These axes can sometimes be thought of as latent variables. For example the leaf economics spectrum (LES) is a principal component axis that describes covariation in a set of plant traits and ranges from a resource acquisitive strategy to a resource conservative strategy (Wright et al. 2004).

Supervised analyses, on the other hand, are appropriate if the goal is to describe a multivariate response to some predictor variable, or to describe multivariate differences between *a priori* chosen groups such as treatment groups in a manipulative experiment (i.e., discriminant analysis). These types of analyses are also not new to Ecology [something about discriminant function analysis and other techinques/ analagous to bi-variate regression]. However, these techniques cannot be used when the number of variables exceeds the number of samples [because…].

Although unsupervised analyses can’t be used to discriminate groups or find relationships in and of themselves, the latent variables they produce are often used in further analyses with the intent of discriminating groups. For example, one might test if two plant species differ in their location along the LES axis with a t-test on principal component axis scores.

For a variety of reasons, ecology researchers often use an unsupervised analysis, such as PCA, to reduce dimensionality, then look for visual patterns in labeled datapoints in a two-dimensional plot of new, latent variable axes. To derive some p-value to report, researchers may perform some kind of univariate hypothesis testing on the values of the datapoints along these new latent variables (e.g. PC axis scores). This can result in complicated interpretation of ecologicaly meaningful results because a significant effect of a PC axis does not necessarily convey meaning unless the axis iteslf makes good biological sense. Not only does this obscure the interpretation of results, but it can also lead to incorrect conclusions because unsupervised and supervised analyses are fundamentally different. For example, unsupervised and supervised analyses are likely to lead to different conclusions when the response variables that most strongly influence the independent variable don’t contribute much to overal covariation in the dataset, as we will demonstrate below. Additionally, determining which variables best predict the independent variable (whether it be categorical or continuous) is complicated in unsupervised analyses like PCA since the axes that best explain the independent variable are likely not the linear combination of variables that best explain the independent variable. Therefore, researchers often use post-hoc univariate tests to determine which variables are driving the relationship after seeing separation in PCA space, unnecessarily inflating type I error.

Partial least squares regression (PLS, also called “projection to latent structures”) and its discriminant analysis extension (PLS-DA), are supervised statistical techniques that work on datasets where the number of variables is greater than the number of samples. PLS was first described in [YEAR, citation] and has since gained popularity in metabolomics[citation], a field that regularly deals with datasets with many more variables (metabolites) than samples. Several statistical software packages have been developed around this technique, specifically for analyzing metabolomic data [SIMCA and metaboanalyst.com]. PLS and its extentions have been adopted by many chemical ecologists[citations], but the usefulness of these techniques is not limited to metabolomic data and is an appropriate approach for answering many ecological questions.

In this primer we intend to demonstrate advantages of PLS over dimensionality reduction followed by univariate hypothesis testing, discuss model validation and hypothesis testing with PLS including important caveats, and demonstrate the use, reporting, and interpretation of PLS results on an ecological dataset.

# Methods (briefly)

## Simulated data methods

I generated a multivariate dataset with a random covariance structure. I am not sure how to “customize” this yet. Here’s the code:

library(MASS)  
library(dplyr)

##   
## Attaching package: 'dplyr'

## The following object is masked from 'package:MASS':  
##   
## select

## The following objects are masked from 'package:stats':  
##   
## filter, lag

## The following objects are masked from 'package:base':  
##   
## intersect, setdiff, setequal, union

vars = 50 #how many variables?  
vars.diff = 5 #how many of the vars are going to contribute to differences between groups?  
N = 20 #how many samples?  
seed = 100  
set.seed(seed)  
A <- matrix(runif((vars)^2)\*2-1, ncol=vars)  
Sigma <- t(A) %\*% A  
data3 <- mvrnorm(n = N, mu = rep(0, vars), Sigma = Sigma) %>% as.data.frame()

I then assigned group membership for each row.

data4 <- data3 %>%  
 mutate(group = c(rep("a", nrow(data3)/2), rep("b", nrow(data3)/2))) %>% #adds a column, "group", with a's and b's  
 select(group, everything())

Then I created a second data set that added 5 new variables that discriminated between groups

set.seed(seed)  
# add x variables that are based on existing ones but with differences between groups  
x = 5  
# strength of difference (passed to rnorm())  
mu = 5  
data5 <- data4 %>%  
 mutate\_at(vars(num\_range("V", 1:x)),  
 # if its in group a, add a random number, if group b, subtract a random number  
 funs(D = ifelse(group == "a", . + rnorm(1, mu, 1), . - rnorm(1, mu, 1))))

Then I did PCA and PLS-DA on both datasets to produce the figure below. I tried this with several random seeds to purposefully cherry-pick an example where PCA reveals no separation, but PLS-DA is significant. I then did univariate t-tests for each variable to check that there were no differences just by chance in my totally random dataset.

## Cupcakes vs. Muffins methods

I took a random subsample of 40 recipes (20 muffins and 20 cupcakes) where each variable is an ingredient (in cups per serving) and applied PCA and PLS-DA on it. I tried this with several random seeds to purposefully cherry-pick an example where PCA revealed some separation to compare the results of PCA vs PLS-DA.

I should also do PLS regression on calories per serving and include that instead or in addition to the cupcakes vs. muffins plots.

# Results

## Simulated data set

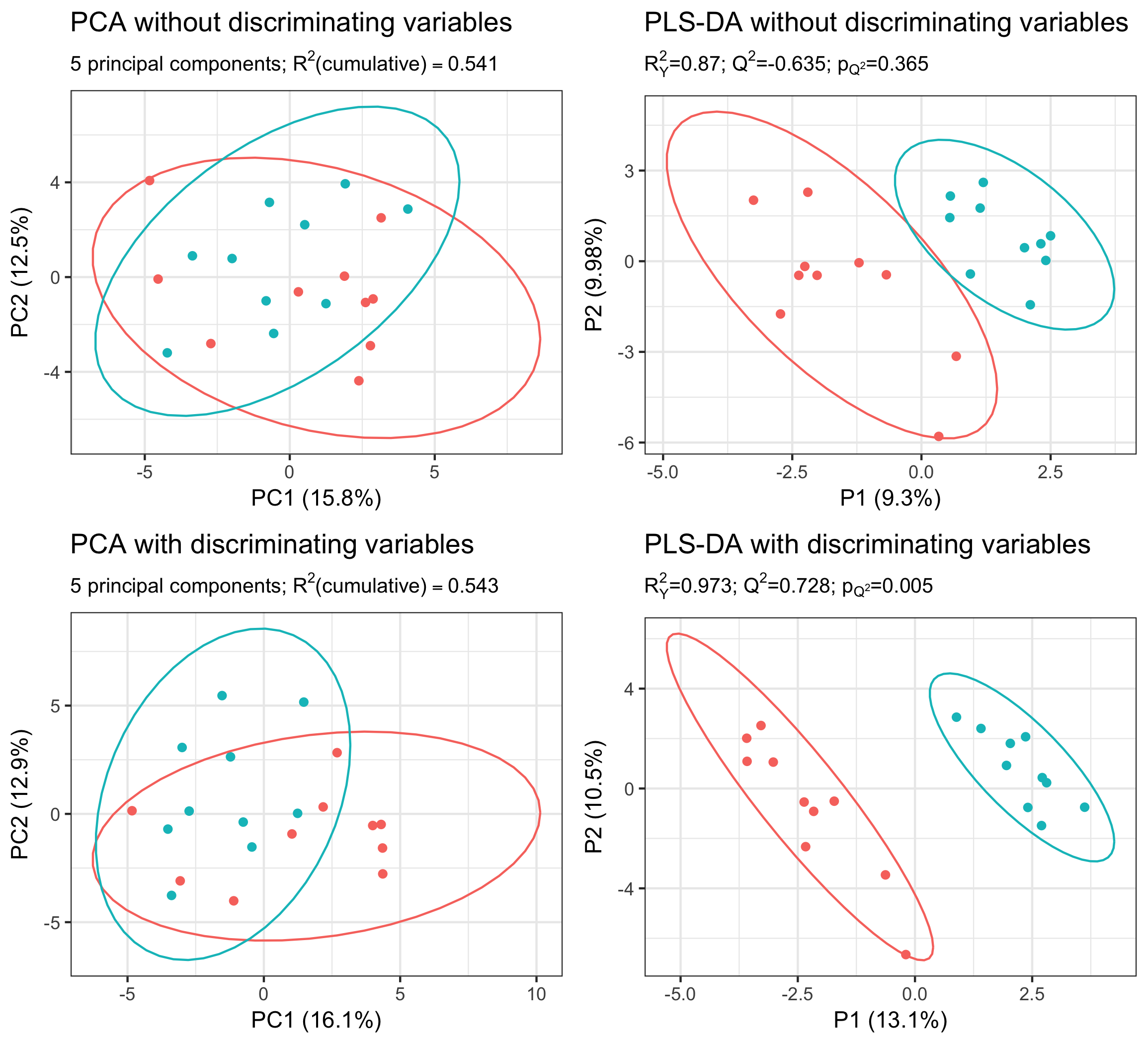


Figure 1: Multivariate analysis of simulated data with random group assignment (A, B) and with 5 aditional variables generated to discriminate between groups (C, D). For PCA plots (A, C), 5 prinicpal components were retained, and the first two principal components are plotted. For PLS-DA plots, the first two predictive axes are plotted, Q2 values are calculated using 7-fold cross validation, and pQ2 is calculated with 200 permutations. Ellipses represent 95% confidence bounds, parenthetical numbers on axis labesl are the percent of total variation explained by the axis. Note, in figure B, the PLS-DA is clearly not a good model due to low Q2 and a high p-value. We recommend not including a PLS-DA plot for non-significant results in a publication.

The addition of 5 discriminating variables has a negligible effect on the PCA. There is still essentially no separation between the two groups along either PC1 or PC2. However, the effect of these discriminating variables on the PLS-DA is apparent both in the visual separation between groups as well as the and values. The PLS-DA on completely random data also demonstrates the tendency of PLS to overfit. Without any cross-validation, one might conclude that the two groups were different, however the extremely low and high p value from this model indicates that this separation is due to chance. Without reporting these cross-validation measures, the PLS-DA plot alone would be extremely misleading. We therefore recommend that plots of non-significant PLS models not be included in publications. It’s also worth noting that even though only 5 of 55 variables were created to distinguish the groups, the first predictive axis of the PLS-DA on the full data set describes 13.1% of the total variation in the data. In addition, the VIP scores of 5 non-discriminating varibles were over 1. Maybe VIP scores alone shouldn’t be used to determine importance of variables. False-discovery-rate adjusted t-tests more accurately identify distinguishing variables in this case.

Table 1: Table of variables with variable importance in projection (VIP) scores greater than 1. Means for each group are reported for each variable, and p-values from t-tests are reported as raw (p) and false discovery rate adjusted (FDR adjusted). Variables ending in “D” were those generated to discriminate between groups.



## Cupcakes vs. Muffins

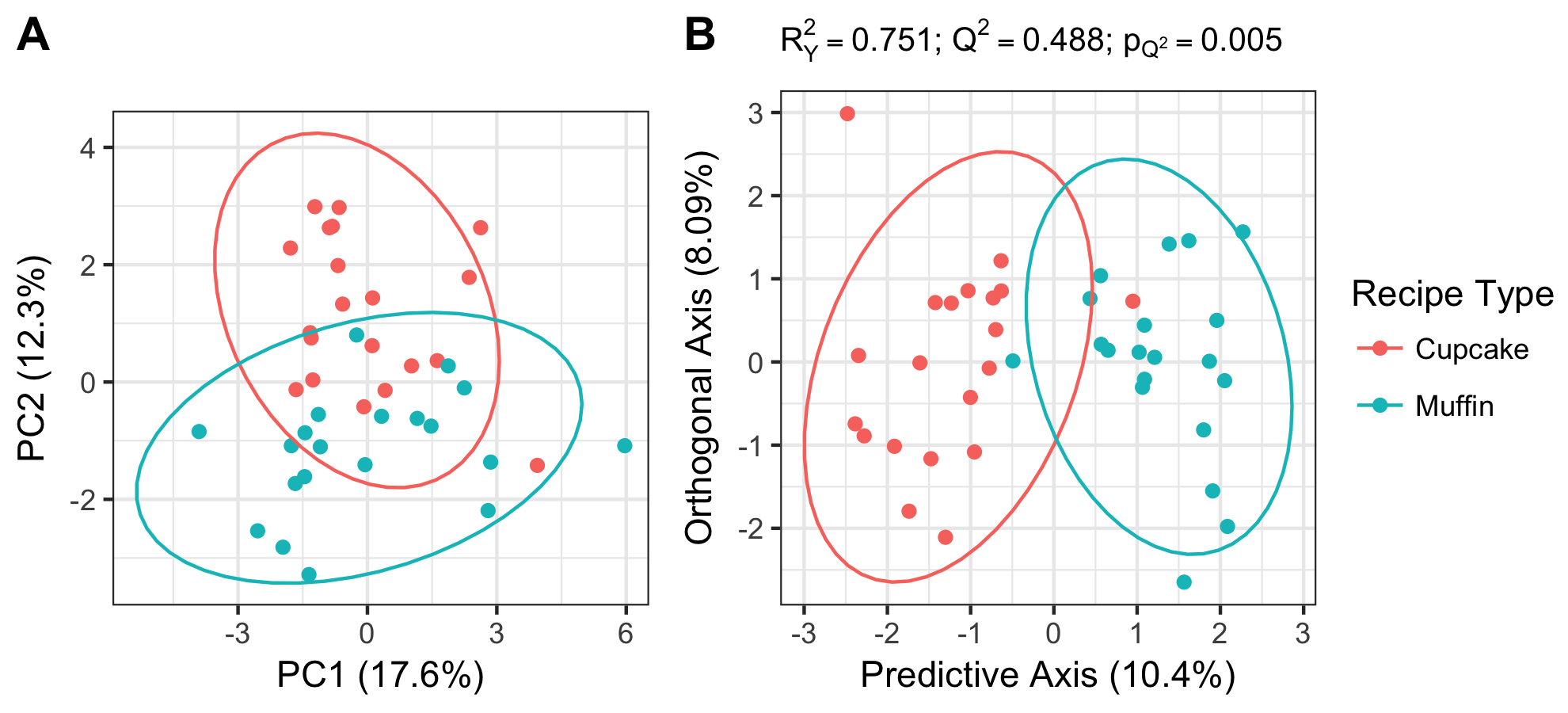


Figure 2: PCA (A) and OPLS-DA (B) on 20 cupcake and 20 muffin recipes.

Table 2: Variable importance in separating muffins from cupcakes. For each variable we report a variable importance in projection (VIP) score, the loading/correlation with the OPLS-DA predictive axis, and the loadings/correlations with the first two PC axes. VIP scores > 1 are generally considered important to separation among groups.



PCA and OPLS-DA both show some separation between cupcakes and muffins, but there are substantial differences in the two methods. First, although PC1 explains the greatest amount of covariation in the ingredients, it does not show any separation between cupcakes and muffins. PC2 shows some weak separation between cupcakes and muffins. This indicates that the variables with the greatest (co)variation in the dataset are not good predictors of the type of baked good. Even when comparing PC2, which separates cupcakes and muffins, with the OPLS-DA predictive axis, there are substantial differences. For example, “unitless” ingredients (e.g. “one sweet potato”, “25 blueberries”) are strongly negatively correlated with PC2 (toward muffins), however, it is not a good predictor of muffins vs. cupcakes as evidenced by its low VIP score and its weak correlation with the OPLS-DA predictive axis. Conversely, “spice” is a good predictor of muffins vs. cupcakes (muffins have more spices than cupcakes) as evidenced by a VIP greater than 1 and a stronger correlation with the OPLS-DA predictive axis, but have a weak correlation with PC2.

# Discussion

Results of simulated data.

Worley and Powers (2016) also compare separation in PCA score space with that in OPLS-DA score space. While the authors show that for OPLS-DA, as model validity decreases, separation of groups in score space is basically unaffected, their suggestion that PCA is a reliable indicator for OPLS-DA reliability is not necessarily true under all situations. When discriminating variables are a small portion of the total number of variables, PCA is especially unreliable at detecting separation. That is, the variables responsible for the greatest amount of variation (explained by PC axis 1) are not necessarily the same variables that reliably distinguish groups. In this situation, poor separation in PCA space is not an indicator that real differences between samples don't exist. In this case, (O)PLS-DA may still be able to detect differences between groups and identify the distinguishing variables with high VIP scores reliably.

* Discuss (mention?) variations on PLS and their advantages and disadvantages
  + Orthogonal PLS (OPLS, OPLS-DA)
  + Sparse PLS (sPLS), implemented in mixOmics. Does variable selection at the same time as PLS
  + Multi-level PLS, implemented in mixOmics. Allows for nested and repeated-measures designs.
* What to report/not report for PLS
  + MUST report some measure of cross-validation, ideally multiple measures
  + PCA is a good companion, but just because PCA shows no separation, doesn’t invalidate PLS-DA (contrary to recommendations of Worley and Powers (2016)).
  + What % total variation explained by predictive axes? If it’s small, that’s an indicator that discriminating variables don’t vary much, but might still be important
  + DON’T report plots of non-significant PLS-DA. Visual separation in the plot is misleading!
  + NEVER do univariate tests on PLS axis loadings. Use cross-validation to determine if model is significant and explanatory.
* Who should use PLS/PLS-DA? When to use?

# Questions I have (that I should find answers for)

* What, if any, are the assumptions of PLS?
* How robust is it to departures from these assumptions?
* How exactly is Q2 calculated (from cross-validation, but what does it mean)? What is RMSE?
* What are the alternatives to permutation testing to get p-values implemented in other packages. CV-ANOVA?
* What exactly is VIP? How is it related to the s-plot in SIMCA?
* How do I alter the covariation of a randomly generated dataset? (what’s a covariance matrix?)

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