# Metabolomic responses of a native and nonnative shrub layer species to deer exclosure and herbivory in suburban forests

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# urban/suburban/exurban ecology

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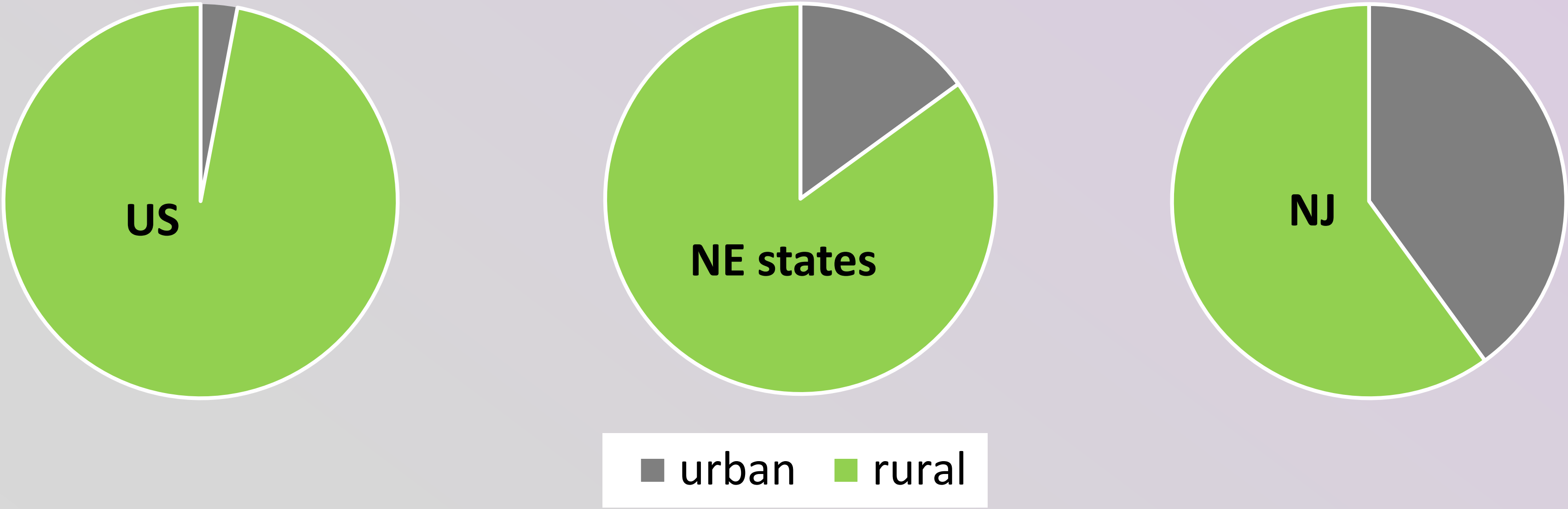
## LAND  (2010 US Census data):



# suburban ecology

Charley Harper

# overabundant deer


wildnewjersey.tv


Hal Korber/PGC Photo


www.al.com

# overabundant deer

Princeton, NJ:
45 deer / $km^2$

Hopewell, NJ:
32 deer / $km^2$

# overabundant deer

## Known consequences for plants of herbivory by deer

For individuals:

- Loss of winter buds
- Loss of flower buds and fruits/seeds
- Loss of foliage
- Death of seedlings
- Variation due to deer food preferences

For communities:

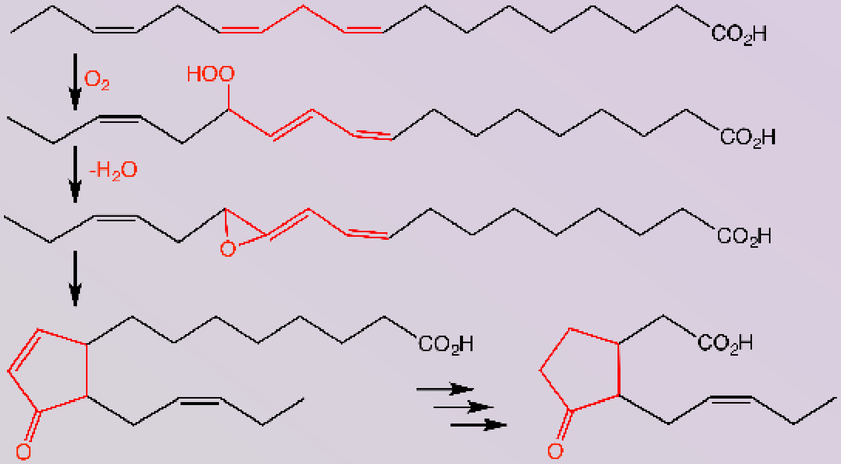
- Reduced abundance in the understory vegetation
- Reduced diversity in the understory vegetation
- Shifts in understory community structure and potential for shifts in canopy species

. . . and consequences for higher trophic levels

# overabundant deer

## An unknown consequence for plants of chronic herbivory by deer: the metabolomic response

Wikipedia (Creative Commons)



jasmonic acid biosynthesis (Wikimedia Commons)

# plant metabolomics

Folen dre – Wikipedia (Creative Commons)

- Plants produce thousands of phytochemicals, or secondary metabolites
- Many act as plant responses to stress and as defenses against herbivores, including
  - nitrogenous compounds: alkaloids, cyanogenic glycosides, glucosinolates, benzoxazinoids
  - terpenoids
  - phenolics
- The functions of many secondary metabolites are unknown
- With metabolomic techniques, we are now able to obtain a profile of the metabolites produced by a plant
- We can design experiments to investigate plants' production of metabolites to compare species, populations, or individuals under differing conditions related to herbivory

# eco-metabolomics of woody plants living with chronic deer herbivory

We can investigate the metabolomic profiles of plants under differing ecological conditions in natural communities

**Research questions:**

- Does exposure to overabundant deer alter woody plants' metabolomic profile?

- If so, does it indicate increased production of anti-herbivore chemical defenses, and which ones?

- Does the response differ among species, especially among native versus nonnative invasive species?

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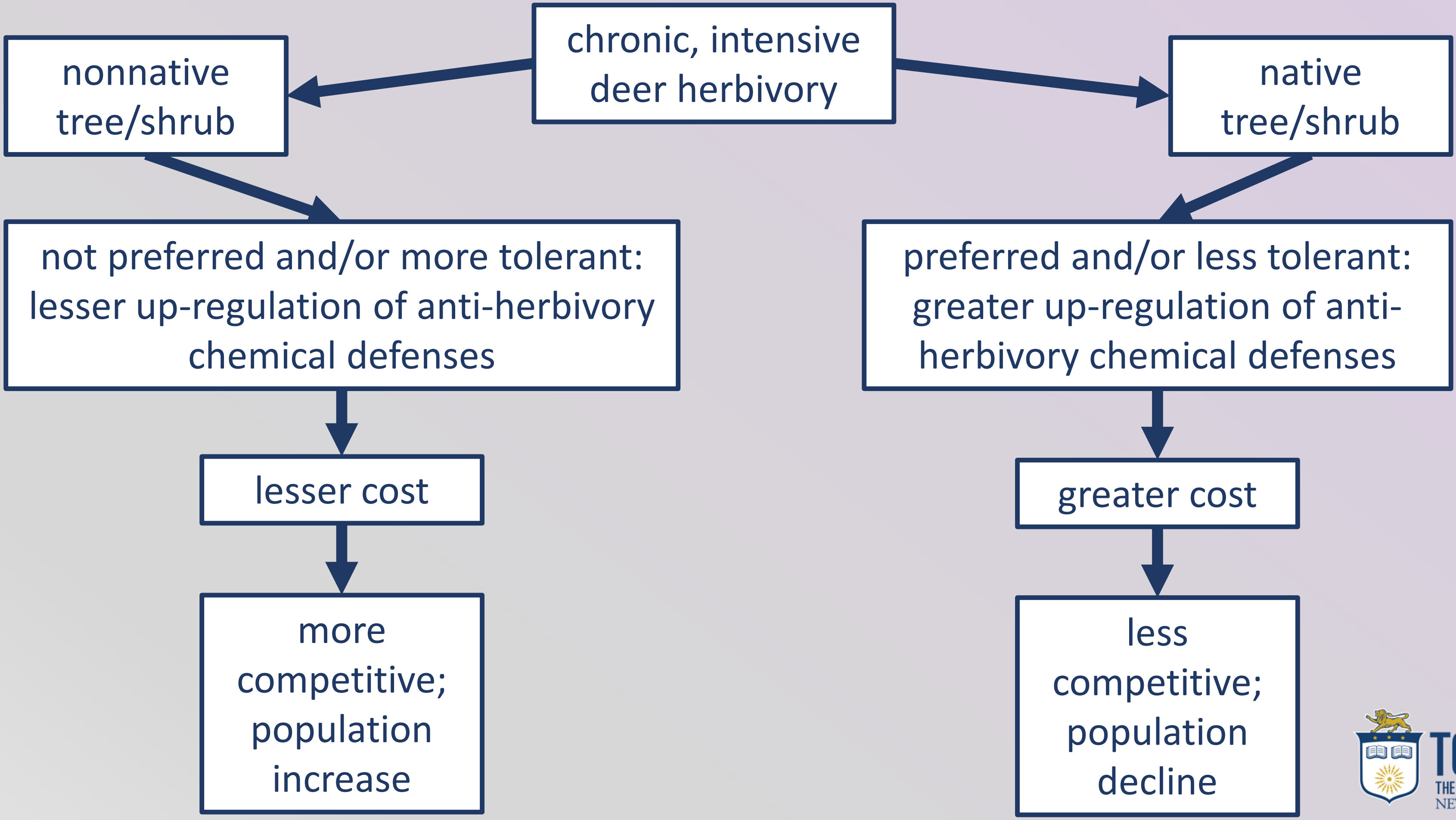
# eco-metabolomics of woody plants living with chronic deer herbivory

## A conceptual model of potential implications for invasion ecology



chronic, intensive deer herbivory

nonnative tree/shrub

not preferred and/or more tolerant: lesser up-regulation of anti-herbivory chemical defenses

lesser cost

more competitive; population increase

native tree/shrub

preferred and/or less tolerant: greater up-regulation of anti-herbivory chemical defenses

greater cost

less competitive; population decline

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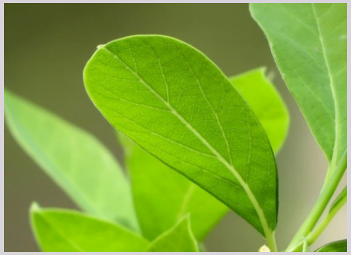
# eco-metabolomics of woody plants living with chronic deer herbivory

Our approach:

- One forest – Herrontown Woods Arboretum, Princeton, NJ
- A deer exclosure experiment:
  - 16 $m^2$ plots (37 total), half randomly assigned to a fencing treatment in spring 2013
- Two focal, woody species in a subset of the plots, both fenced and unfenced:
- A native – spicebush (*Lindera benzoin*)



- A non-native invasive – multiflora rose (*Rosa multiflora*)

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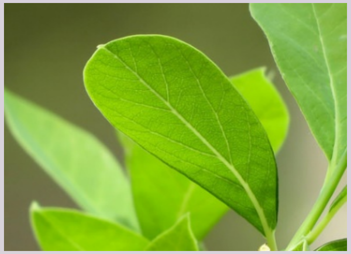

# collecting

- All foliage samples collected 27 July 2018
- From 12 or 14 plots per species
- Alternated fenced and unfenced plots
- Noted presence/absence of recent deer browse on unfenced plants
- Collected from three plants per plot, pooled the tissue
- Placed into liquid N within 30 sec
- Stored at -80 C until shipped to Boyce Thompson Institute on dry ice

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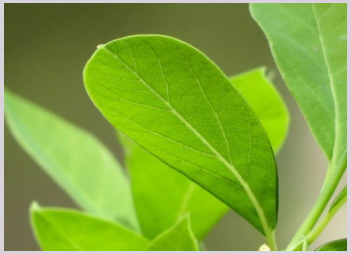
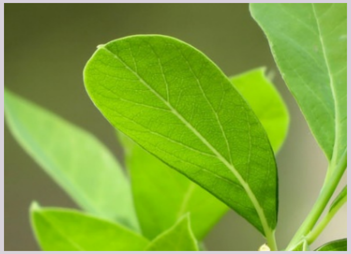


# extraction

- Ground tissue to fine powder in liquid nitrogen
- Vortex-mixed 100 mg of the powder with cold chloroform:methanol:water (1:2:1) buffer for 20 minutes
- Centrifuged at 15000g for 20 minutes
- Transferred clear supernatant to new tube
- Lyophilized until dry
- Resuspended in 70% methanol (in water, v/v)
- Centrifuged as above; retreived clean supernatant



# analysis

- Used the final supernatant
- Untargeted metabolomic analysis by LC-MS/MS (liquid chromatography mass spectrophotometry)
- Spectral data of ~6000 metabolites were converted to mzXML files and pre-processed using R packages XCMS and CAMERA
- Statistical analyses performed on Metaboanalyst

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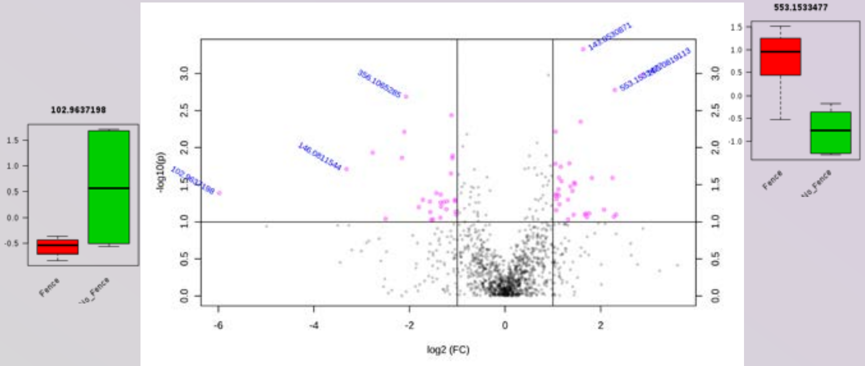
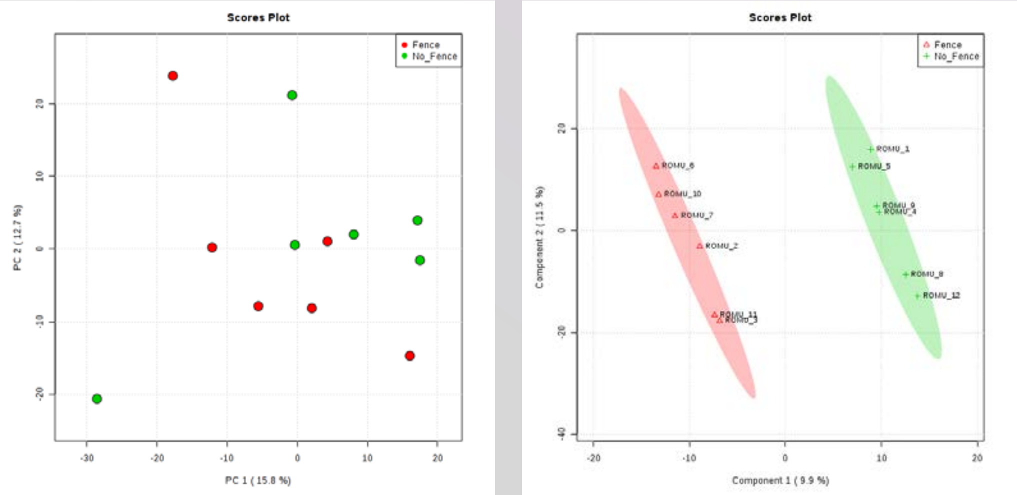
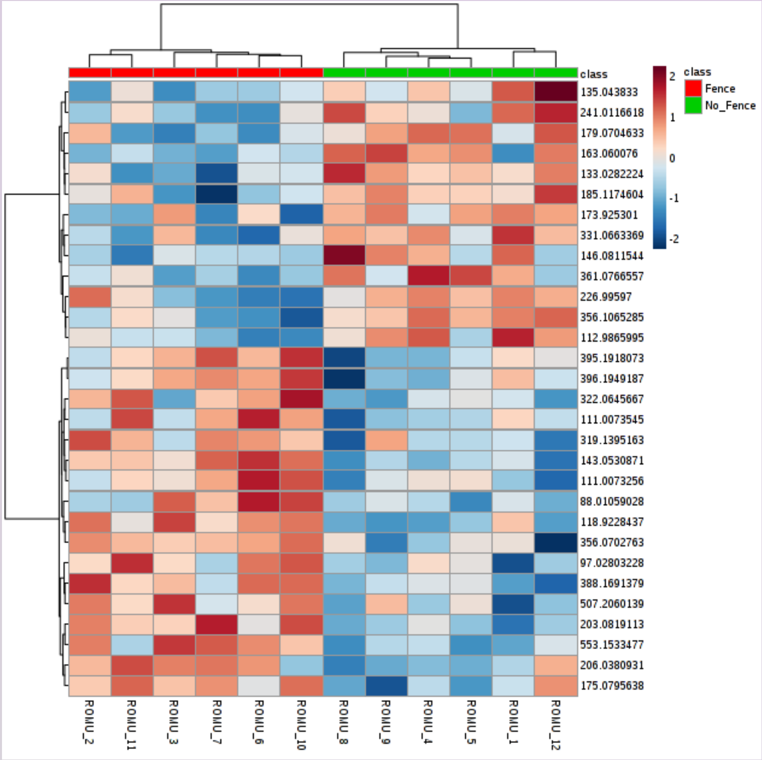
# *Rosa multiflora: fenced vs. unfenced*



**Volcano plot** compares the metabolites in the two groups and identifies the metabolites that differed significantly and that had a fold change of 2 or more (colored dots)

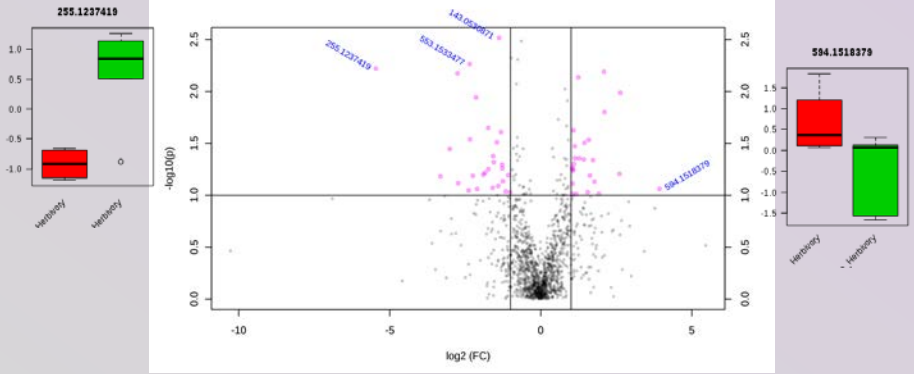


**Principal Component Analysis** (left) and **Partial Least Squares Discriminant Analysis** (PLS-DA) depict the the relationship among the samples based on all the metabolites. Samples that cluster together on these plots have more similar metabolite profile



**Heat map** shows the pattern of accumulation of the top 30 significantly (P <0.01) different metabolites among the samples. Note that, as expected, for each of these metabolites, the pattern observed here seem to agree with the results of the PLS-DA analysis. The numbers on the right are the masses of the metabolites.

# *Rosa multiflora: fenced vs. browsed*



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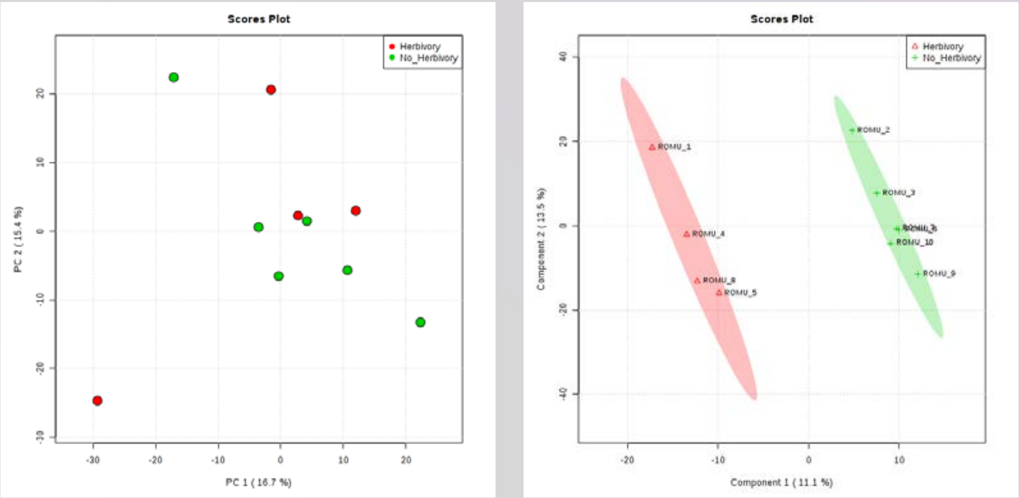

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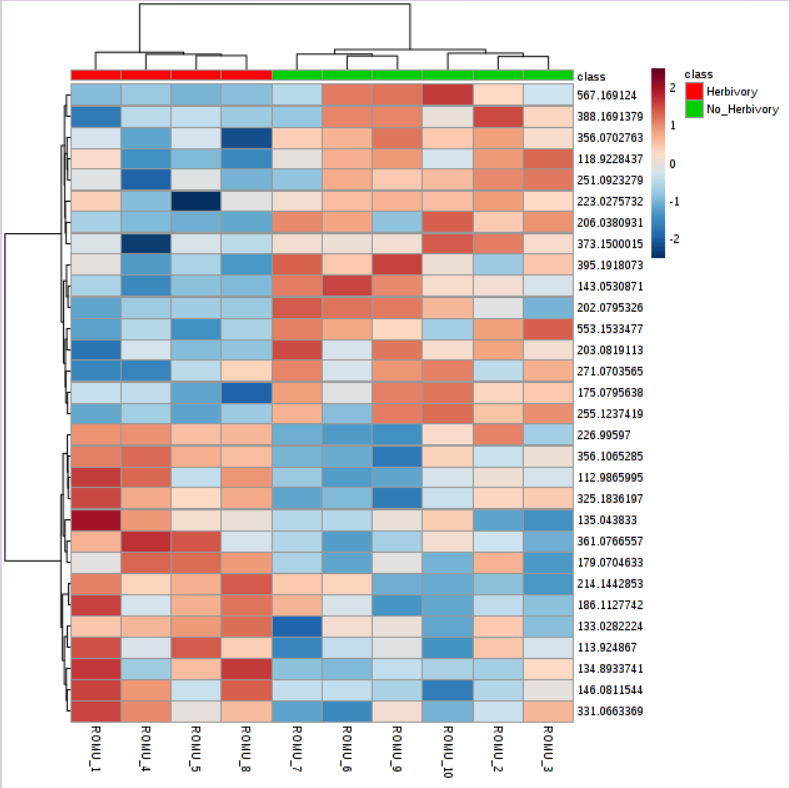

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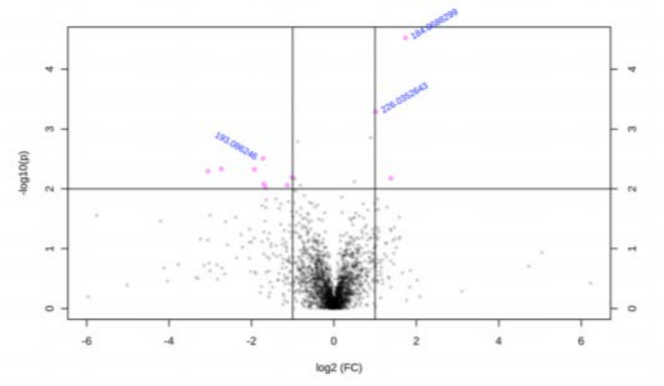

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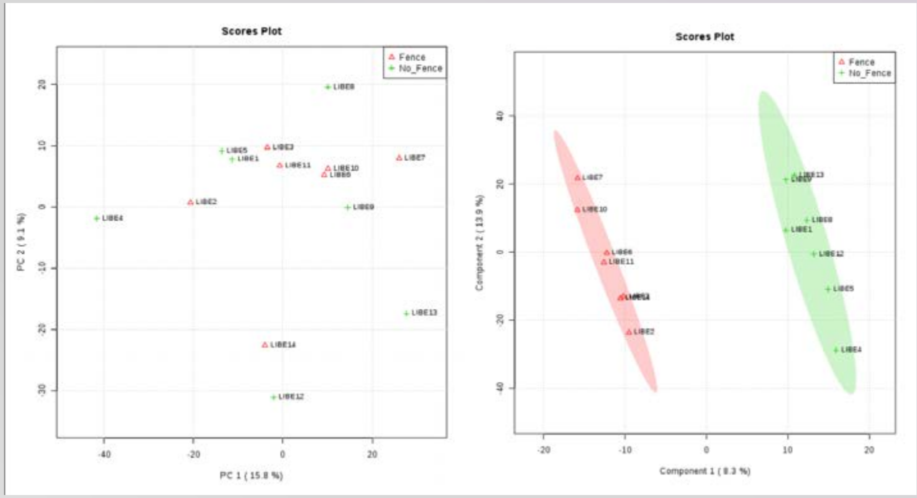

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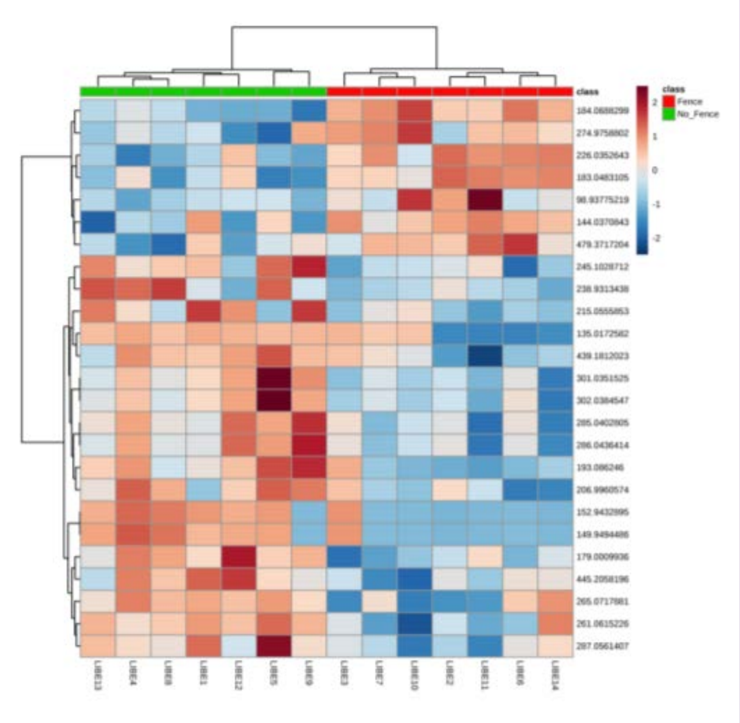

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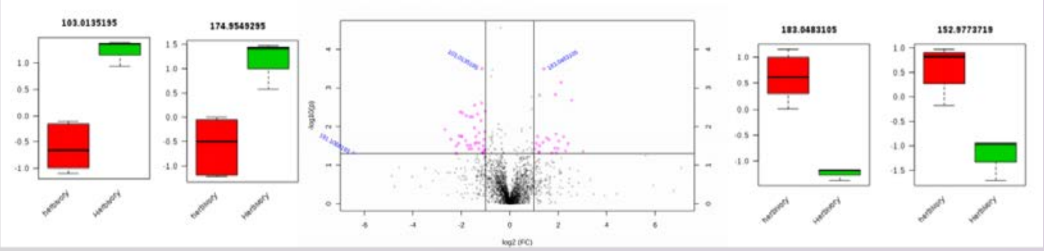
# *Lindera benzoin: fenced vs. browsed*



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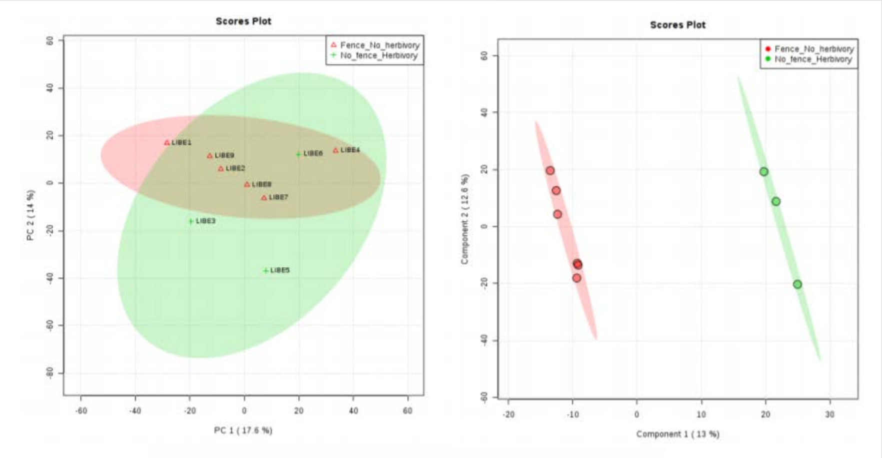

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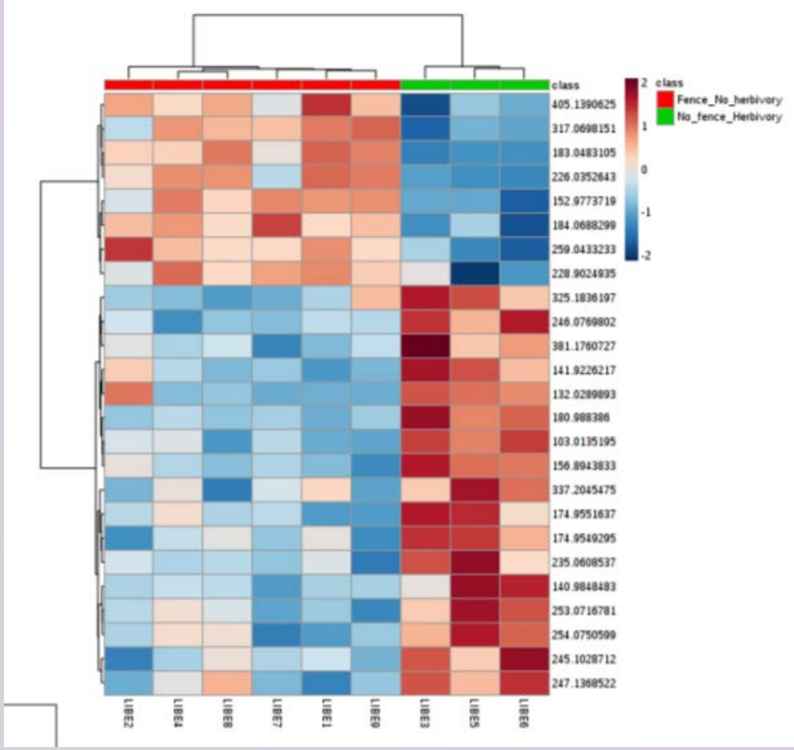

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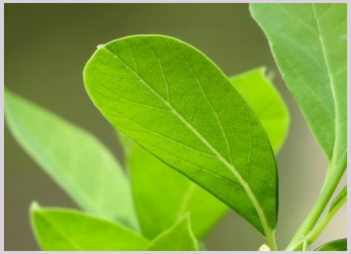
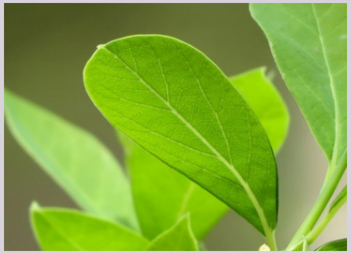


# conclusions

- For both, the metabolomic profiles were different between fenced vs. unfenced plots and for plants with and without browse.
- Despite the inherent qualitative difference in metabolite accumulation between species, we found responses predicted by common metabolic pathways, including the trans-farnesyl diphosphate (FPP) biosynthesis pathway, which plays an important role in sesquiterpene and sterol synthesis and affects jasmonic acid mediated defense responses.
- Chronic exposure to deer browse appears to cause metabolome-wide changes in plants, including defense-related metabolites.
- Further study is now needed to determine how common these changes are among species, and which metabolites are most affected.



# thanks

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**Boyce Thompson Institute**

Permission to conduct research

Mercer County Parks Department

Field work

Daniela Nattes, Elena Nattes, Liz Nemec, Claire Paul
*MANY* students researchers in the Morrison lab since 2013

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