OP-072 [Bee Biology]
Dual effect of S-methyl-L-cysteine sulfoxide from rapeseed nectar on honey bee health

Jaroslav Havlík[1], Saetbyeol Lee[1], Pavel Dobes[2], Pavel Hyrsl[2], Anna Mascellani[1], Martin Kamler[3], Dalibor Titera[3]
[1]Department of Food Science, Czech University of Life Sciences Prague, Prague, Czechia
[2]Section of Animal Physiology and Immunology, Masaryk University, Brno, Czechia
[3]Bee Research Institute, Dol - Maslovice, Czechia

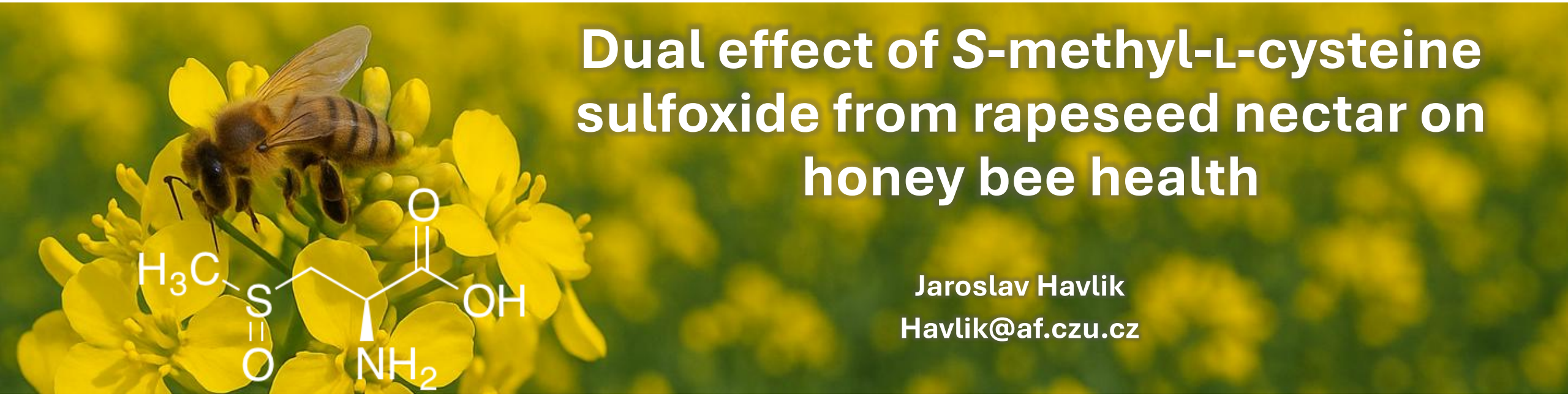
Intensive agriculture affects pollinator health, including honey bees (Apis mellifera). Rapeseed (Brassica napus L.), a widely cultivated oil crop, provides abundant pollen and nectar, and its honey is valued for yield, mild taste and texture. However, rapeseed contains phytochemicals, including S-methyl-L-cysteine sulfoxide (SMCSO). This compound acts as a plant toxin in ruminants, where it is linked to kale anemia, sparking interest in its potential impact on honey bee health.

We quantified SMCSO and its metabolites across seasons, colonies, body parts, and dietary sources using nuclear magnetic resonance spectroscopy. Additionally, a cage-feeding experiment assessed SMCSO's toxicity and effects on antioxidant capacity, antimicrobial activity, proteolytic patterns, and free amino acid profiles.

SMCSO concentrations in the bee gut and body varied across colonies, likely due to dietary preferences, peaking during rapeseed flowering. The thorax showed the highest SMCSO concentration (2.5 µmol/g in foragers), while its main metabolite, 3-methylthiolactic acid sulfoxide, reached 6 µmol/g, particularly in nurses. SMCSO accumulated in tissues, indicating its presence in the body was not solely dependent on immediate gut content. Although nectar contained higher SMCSO concentrations than pollen, both served as significant sources. Short-term SMCSO exposure (1 mg SMCSO given in 1mL of sucrose solution (1:1 w/v; sucrose/water)) had no immediate toxic effects and even enhanced bees' antioxidant capacity. However, prolonged exposure (1 mg/ml SMCSO in sucrose solution (1:1 w/v; sucrose/water) ad libitum for 10 days) led to reduced body weight, particularly in the thorax and head, and altered amino acid metabolism, as reflected in the metabolomic profiles of both the body and gut. In contrast, the control group (sucrose solution (1:1 w/v; sucrose/water)), showed no such effects. This dual effect may explain why rapeseed remains a key forage source despite its phytochemical complexity. Furthermore, SMCSO accumulated in rapeseed honey at significantly higher levels than in other honey types, making it a potential botanical biomarker.

This study provides new insights into SMCSO's role in honey bee health, highlighting both benefits and risks of rapeseed forage. While beekeepers often report variable colony fitness near rapeseed fields, atrributed to pesticides, our findings suggest that SMCSO may also play a role.

APIMONDIA SCANDINAVIA 2025
SWEDEN | DENMARK | NORWAY
23-27 SEPTEMBER
COPENHAGEN



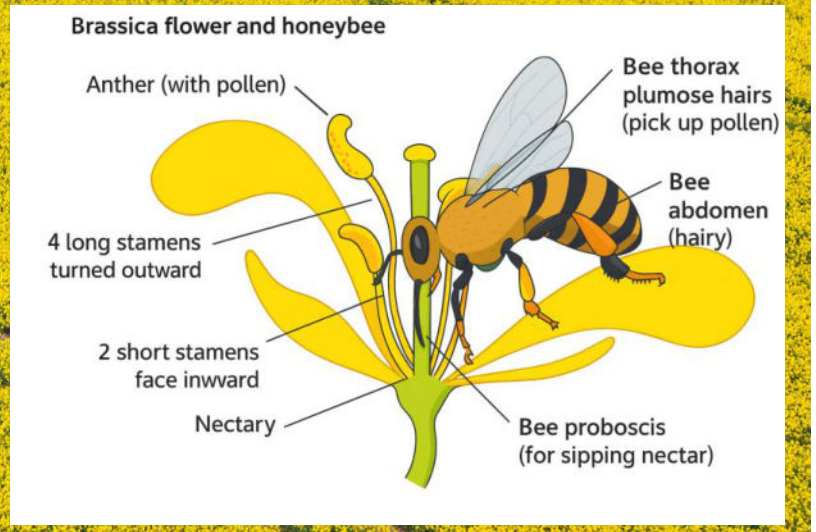
Dual effect of S-methyl-L-cysteine sulfoxide from rapeseed nectar on honey bee health

Jaroslav Havlik
Havlik@af.czu.cz

Faculty of Agrobiology, Food and Natural Resources
University Full of Life

Introduction

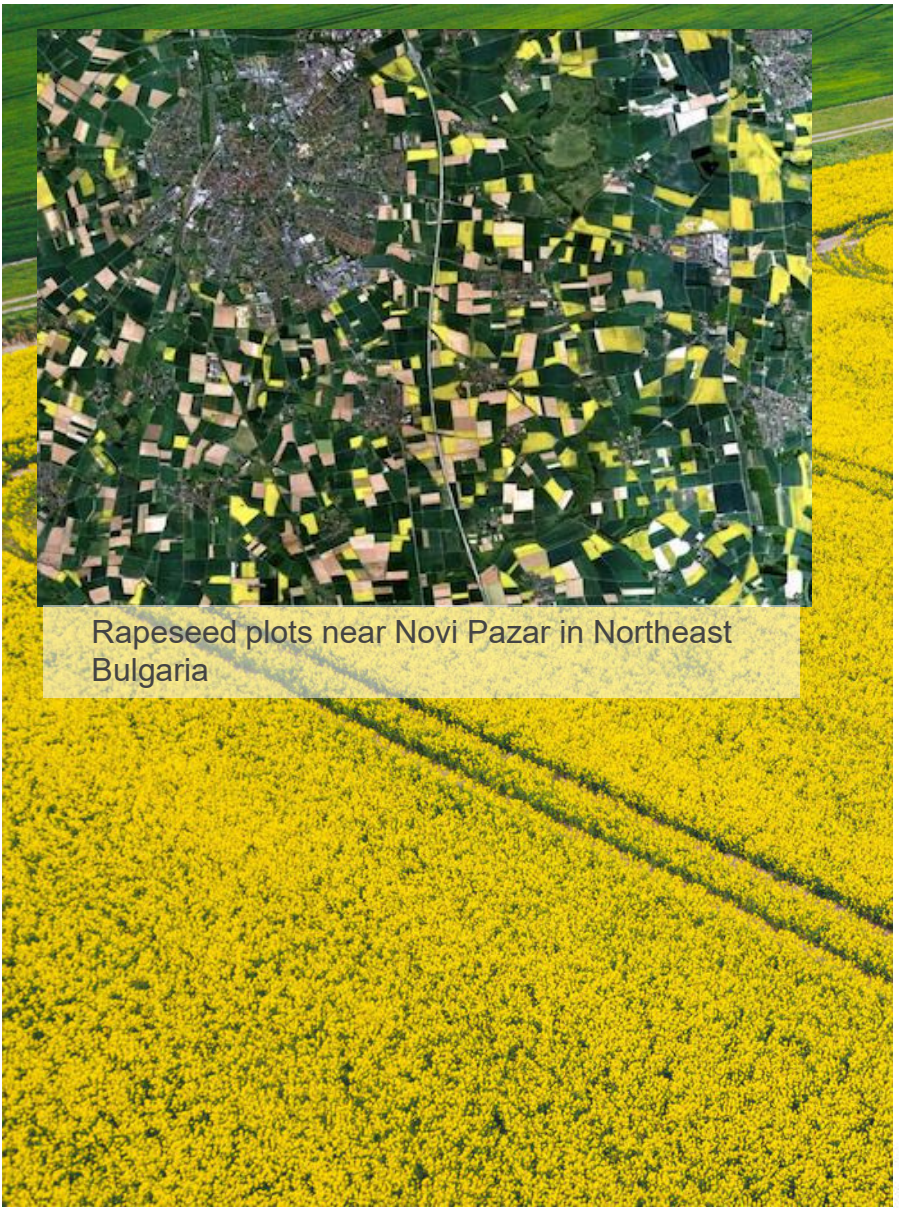
Rapeseed plots near Novi Pazar in Northeast Bulgaria



Based on Abrol (2007) *Adv. Bot. Res.*

- Rape seed, *Brassica napus L.*
- Honeybees play role in increasing yield of rapeseeds, mutual benefits
- **Pollen contains up to 27% protein** and **good amino acid spectrum** (Bryś, 2024, Molecules)
- Pollen very sticky
- **Nectar is glucose–fructose dominant**; sucrose absent or low.
- Pollen supports longevity; bees fed rapeseed pollen lived up to ~2.5× longer than controls. (Schmidt, 1995, J Econ. Ent.)

Introduction



Rapeseed plots near Novi Pazar in Northeast Bulgaria

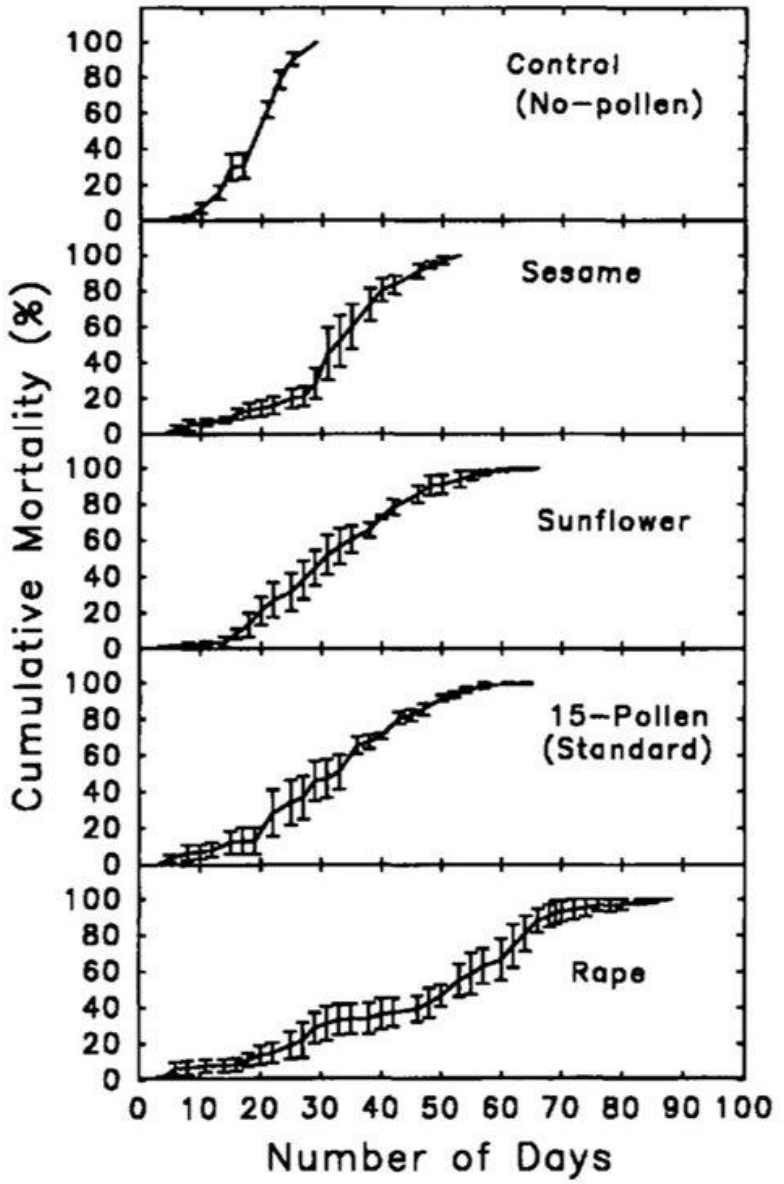


Fig. 2. Longevity patterns of control (no pollen) bees and bees fed sesame, sunflower, rape, or 15-pollen (standard) diets.

- Rape seed, *Brassica napus L.*
- Honeybees play role in increasing yield of rapeseeds, mutual benefits
- **Pollen contains up to 27% protein** and **good amino acid spectrum** (Bryś, 2024, Molecules)
- Pollen very sticky
- **Nectar is glucose–fructose dominant**; sucrose absent or low.
- Pollen supports longevity; bees fed rapeseed pollen lived up to ~2.5× longer than controls. (Schmidt, 1995, J Econ. Ent.)

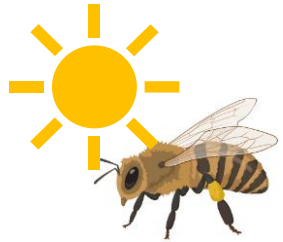
Experiment 1: Seasonal metabolic changes

Summer bees
Short living, 30d

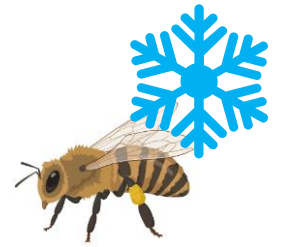
Winter bees
Long living, ~300d

In temperate climate zone

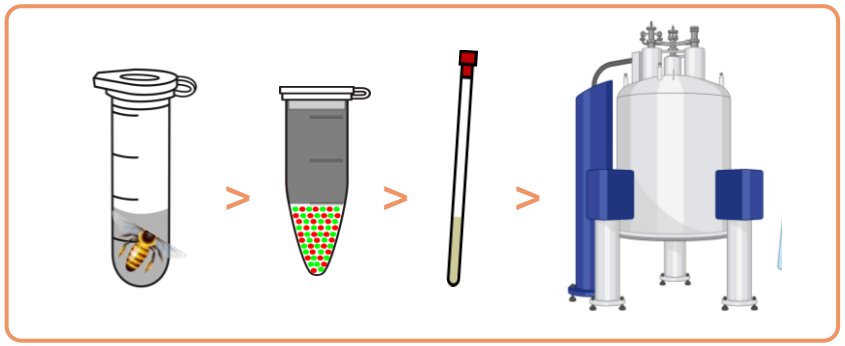
Do summer and winter bees show different physiological features?



Vs.



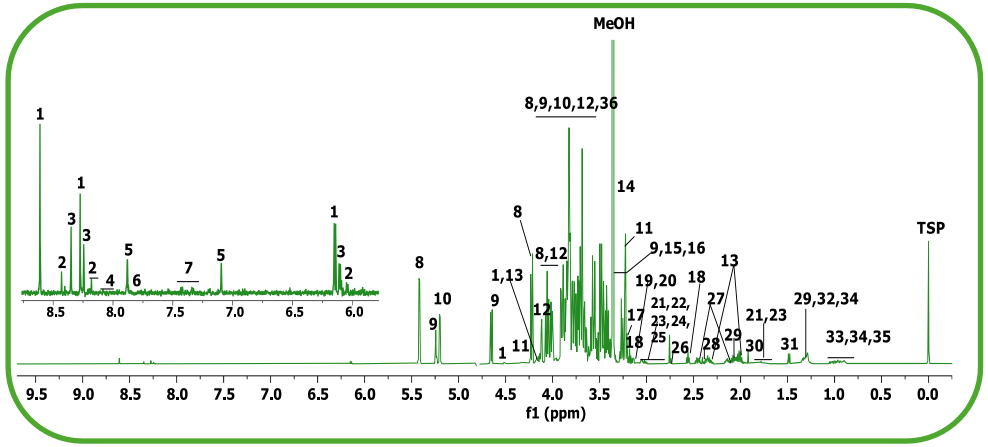
- Hypothesis: There are **metabolic differences within the bodies of these two seasonal phenotypes**. The objective was to distinguish distinct metabolic patterns between these two populations using **^{1}H NMR**
- Summer bees (n = 75) vs. Winter bees (n = 45)
- One apiary, three hives, 11-day old same age
- Multivariate analysis (PCA, OPLSDA), univariate analysis (*t*-test)



Metabolomics

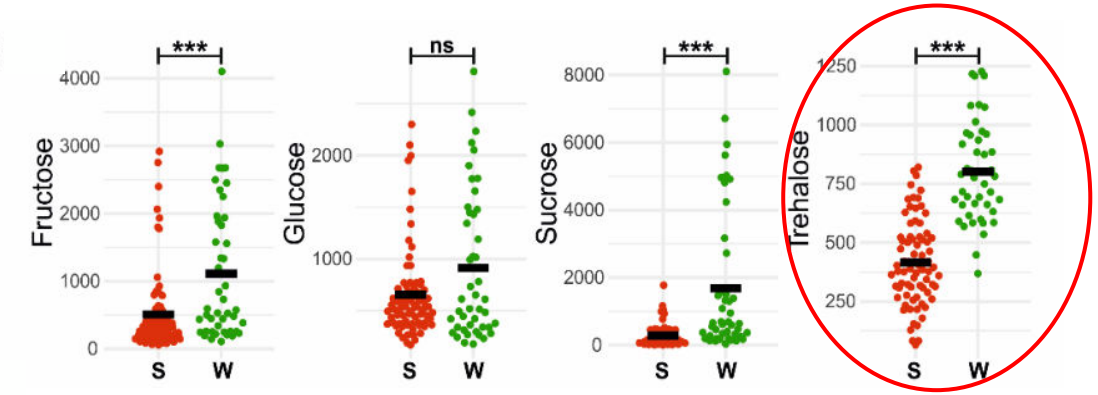
Experiment 1: Seasonal metabolic changes

A total of 36 metabolites were identified & quantified

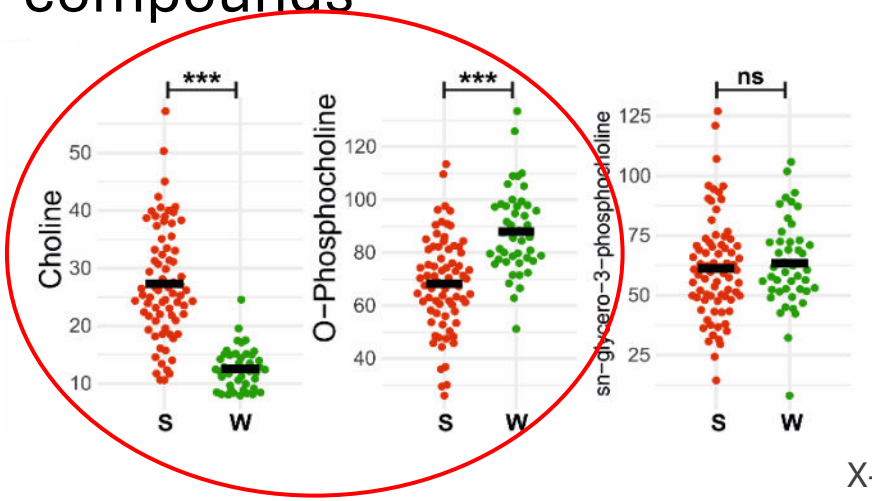


Lee, 2002, Insects

Carbohydrates



Choline-containing compounds

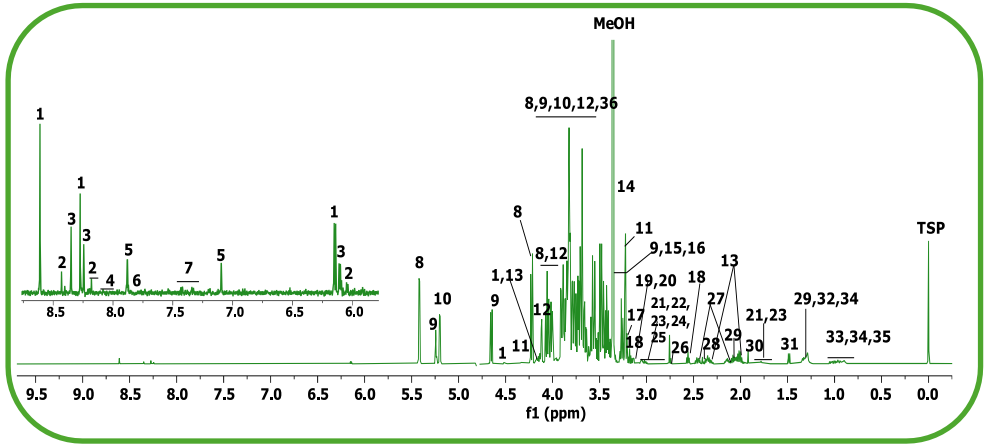


* $p < 0.05$, ** $p < 0.005$, *** $p < 0.0001$, ns = not significant

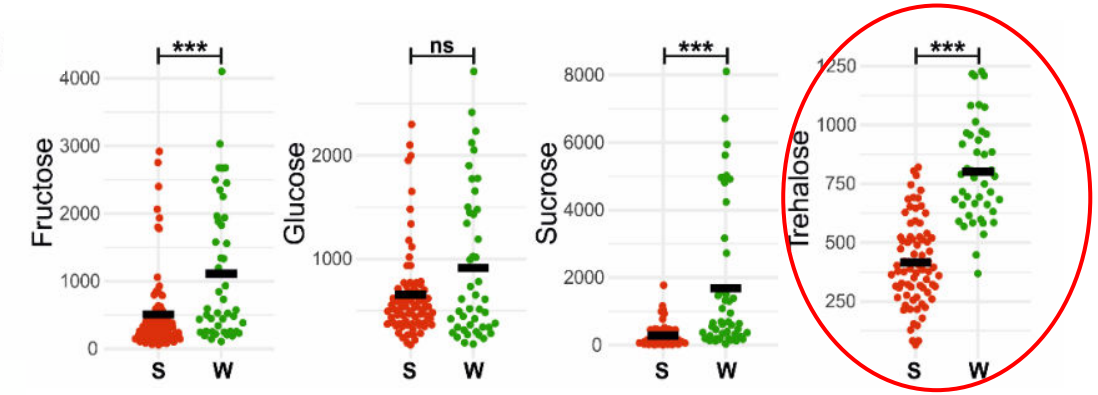
X-axis: the groups summer (red) and winter (green), y-axis: metabolite concentration (ug/bee), black bar: mean

Experiment 1: Seasonal metabolic changes

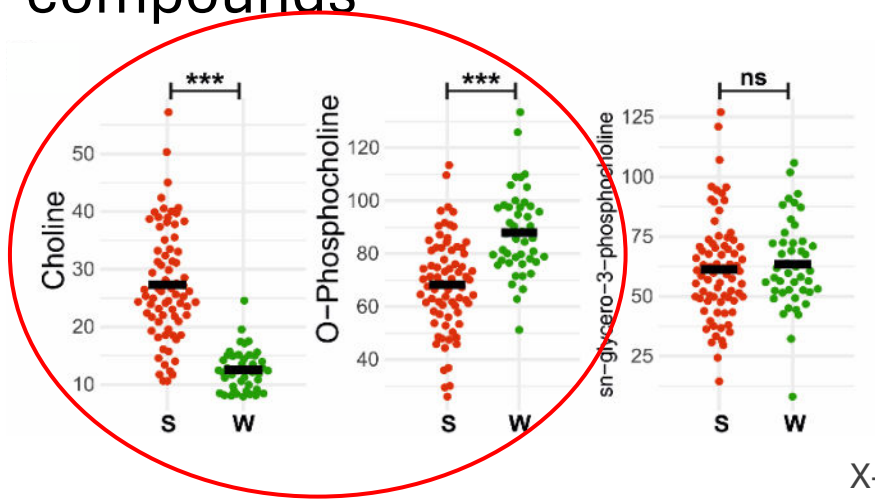
A total of 36 metabolites were identified & quantified

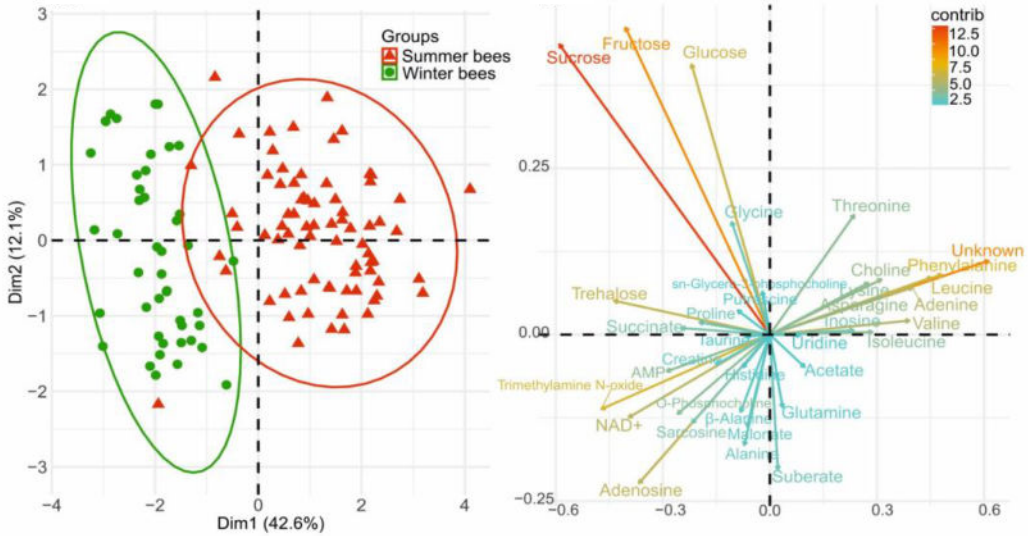


Carbohydrates



Choline-containing compounds





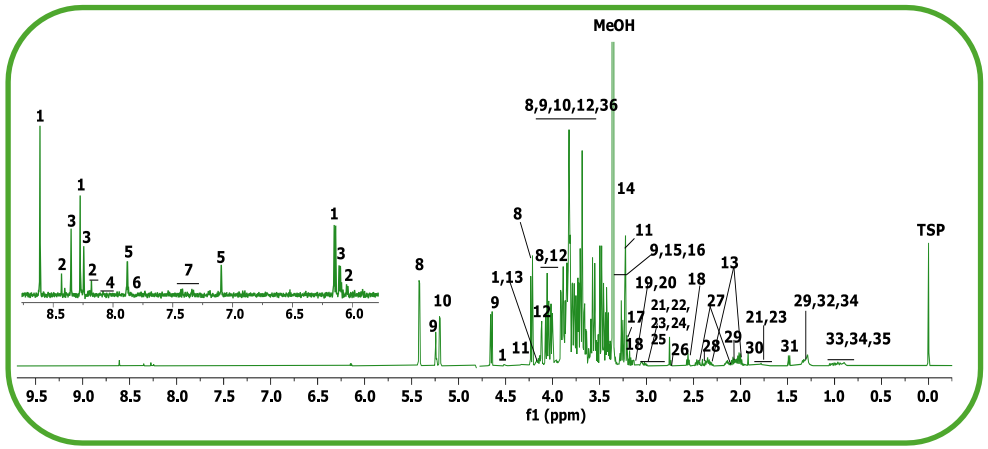
Lee, 2002, Insects

$*\ p < 0.05$, $**\ p < 0.005$, $***\ p < 0.0001$, ns = not significant

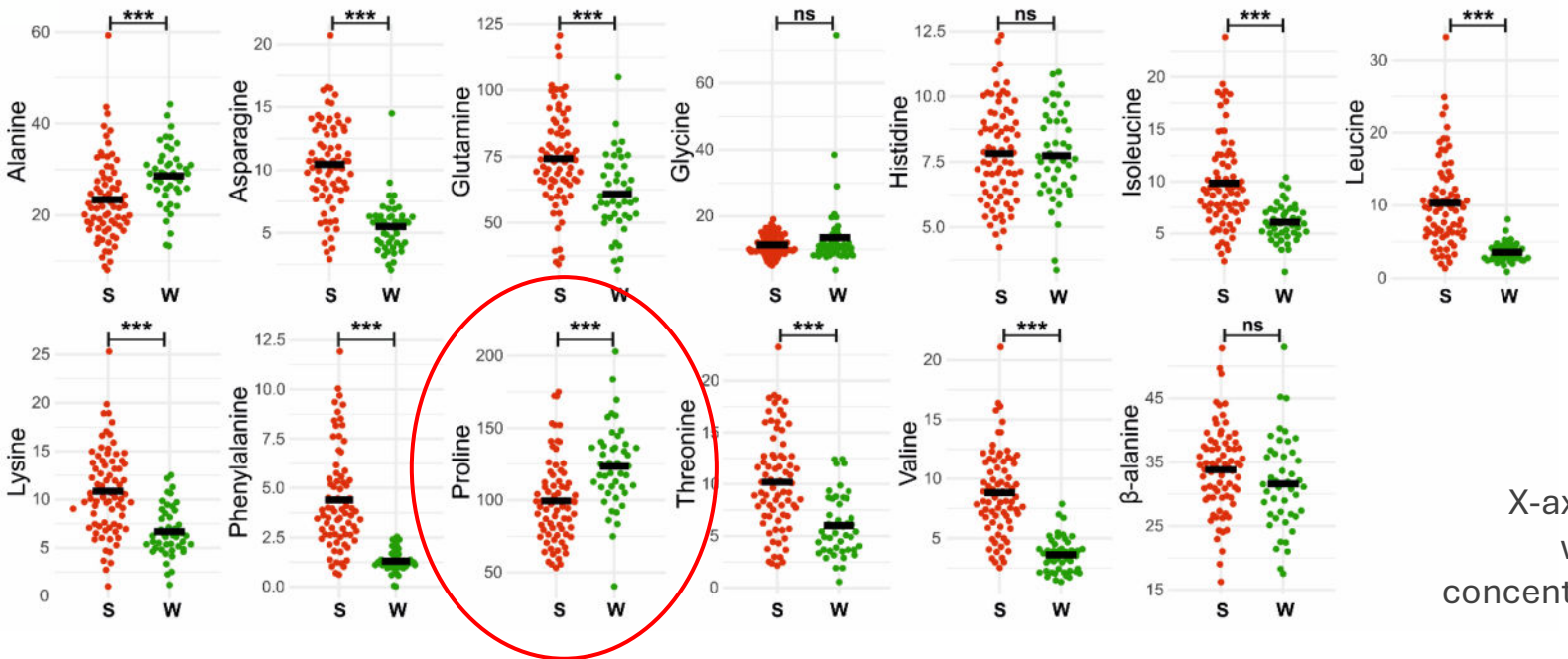
X-axis: the groups summer (red) and winter (green), y-axis: metabolite concentration (ug/bee), black bar: mean

Experiment 1: Seasonal metabolic changes

A total of 36 metabolites were identified & quantified



Amino acids



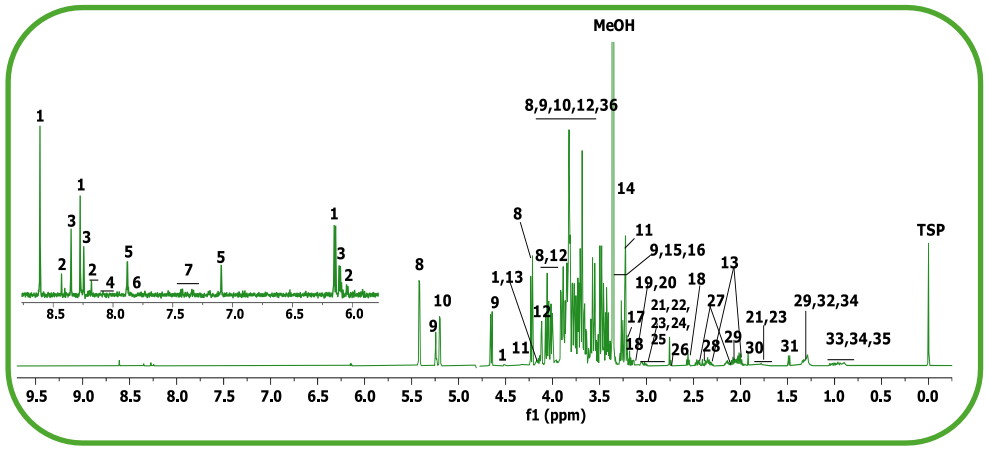
* $p < 0.05$, ** $p < 0.005$, *** $p < 0.0001$, ns = not significant

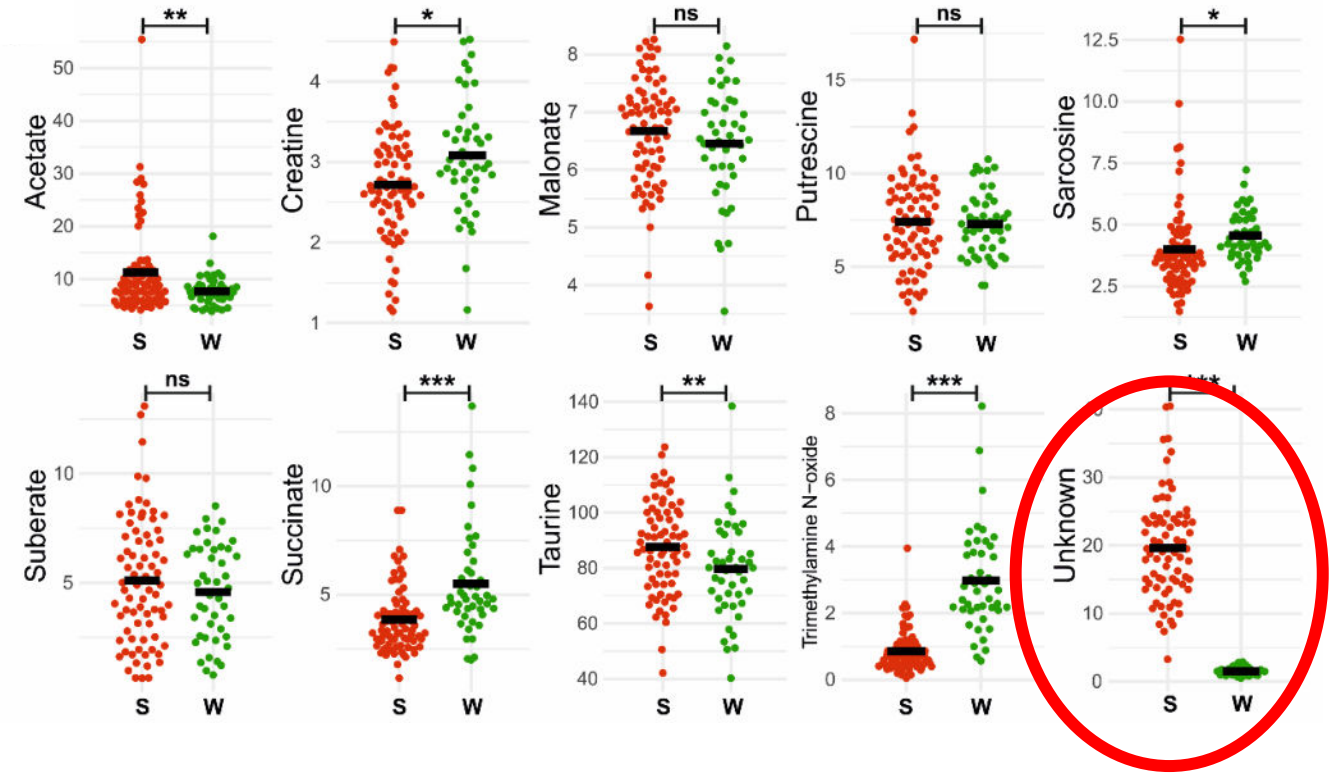
X-axis: the groups summer (red) and winter (green), y-axis: metabolite concentration (ug/bee), black bar: mean

Lee, 2002, Insects

Experiment 1: Seasonal metabolic changes

A total of 36 metabolites were identified & quantified





Other + amines

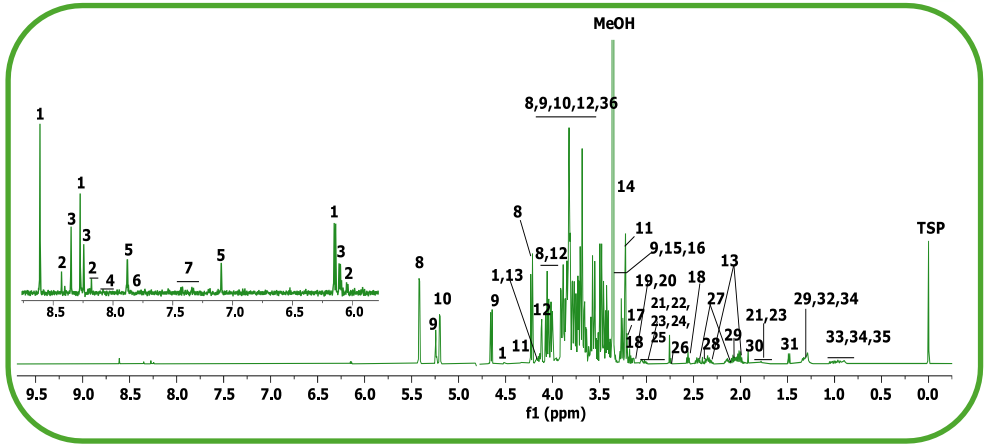
* $p < 0.05$, ** $p < 0.005$, *** $p < 0.0001$, ns = not significant

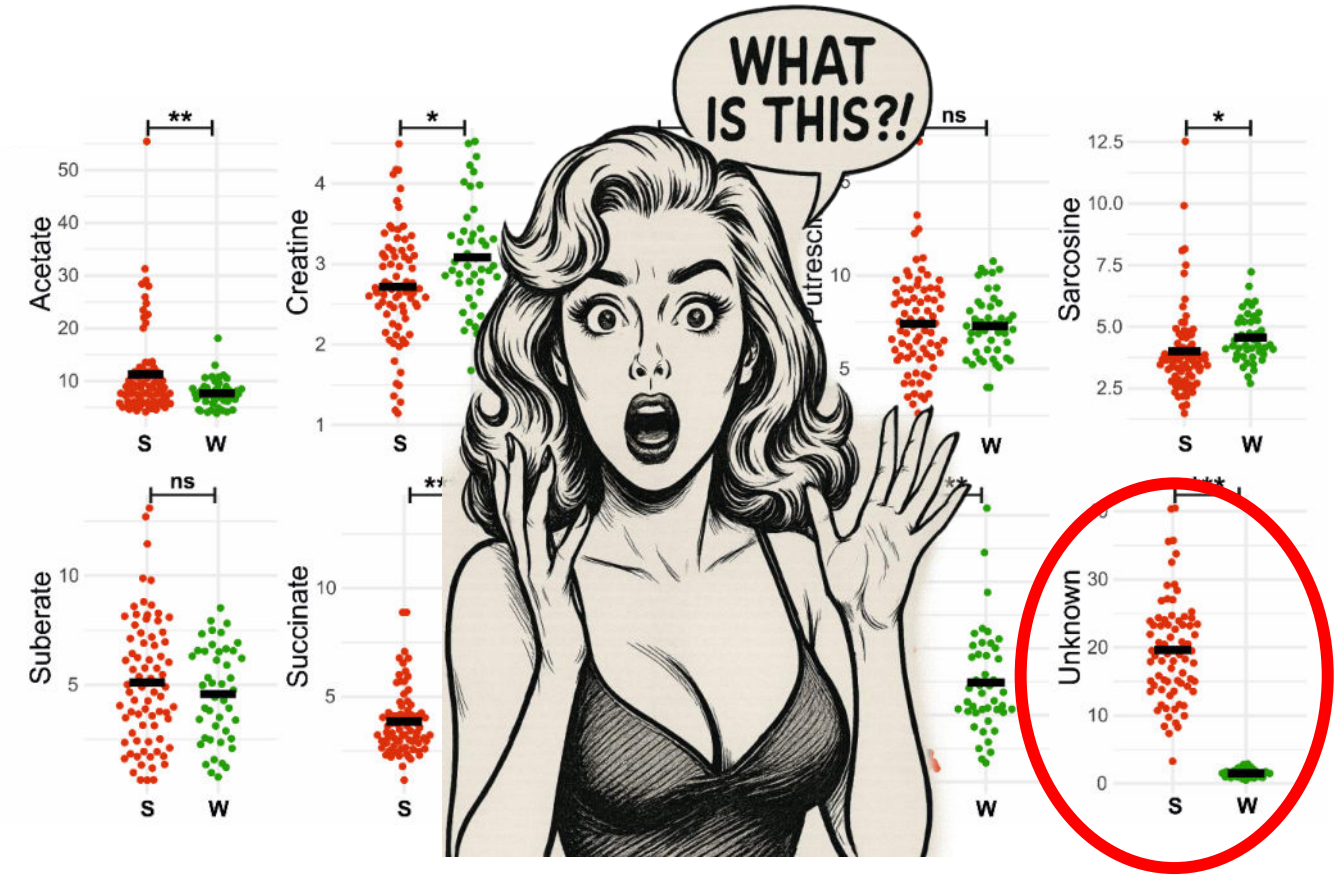
X-axis: the groups summer (red) and winter (green), y-axis: metabolite concentration (ug/bee), black bar: mean

Lee, 2002, Insects

Experiment 1: Seasonal metabolic changes

A total of 36 metabolites were identified & quantified





Other + amines

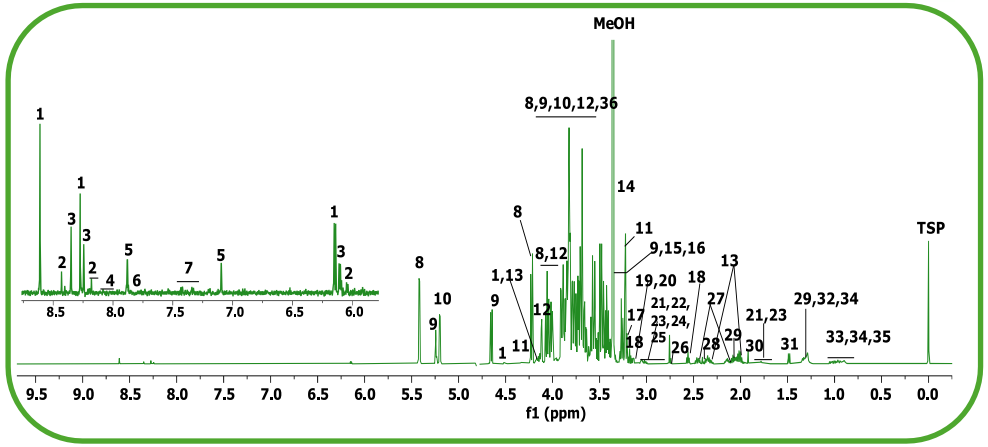
Lee, 2002, Insects

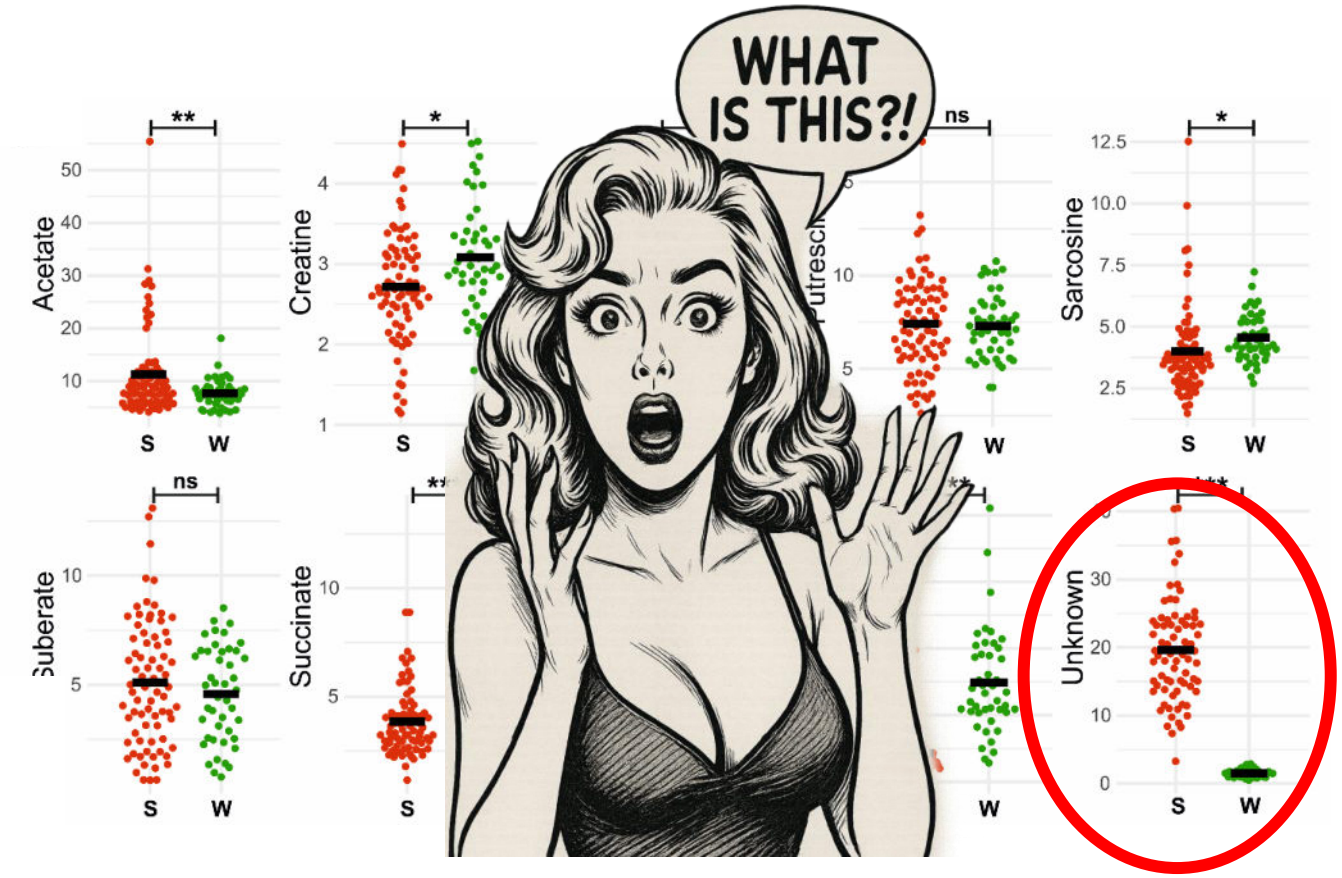
* $p < 0.05$, ** $p < 0.005$, *** $p < 0.0001$, ns = not significant

X-axis: the groups summer (red) and winter (green), y-axis: metabolite concentration (ug/bee), black bar: mean

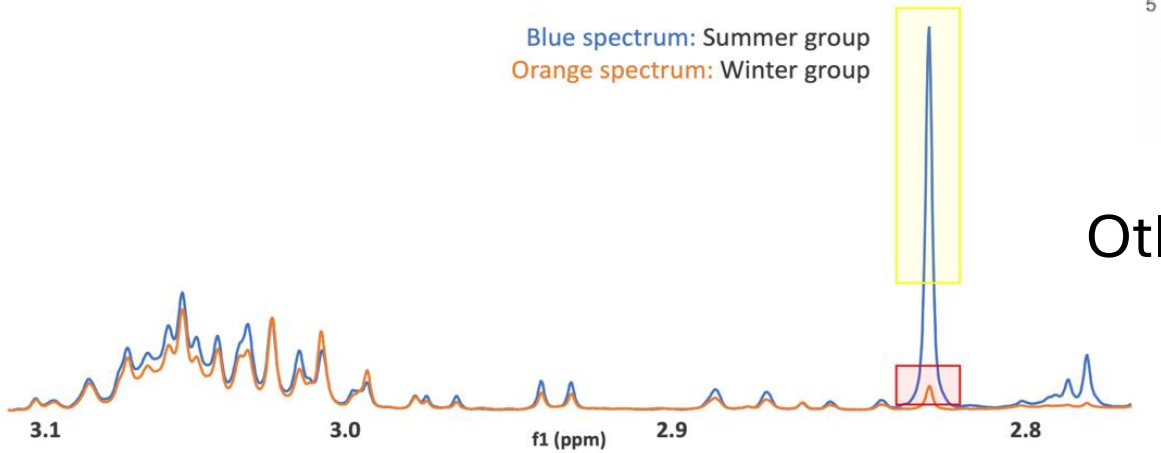
Experiment 1: Seasonal metabolic changes

A total of 36 metabolites were identified & quantified





Other + amines

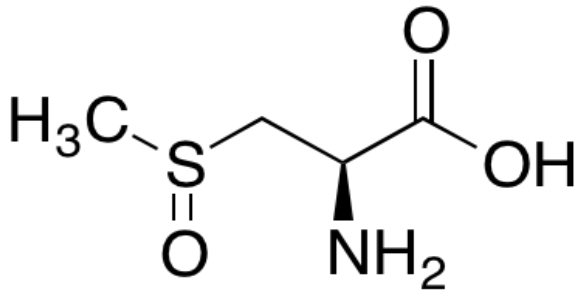


Lee, 2002, Insects

* $p < 0.05$, ** $p < 0.005$, *** $p < 0.0001$, ns = not significant

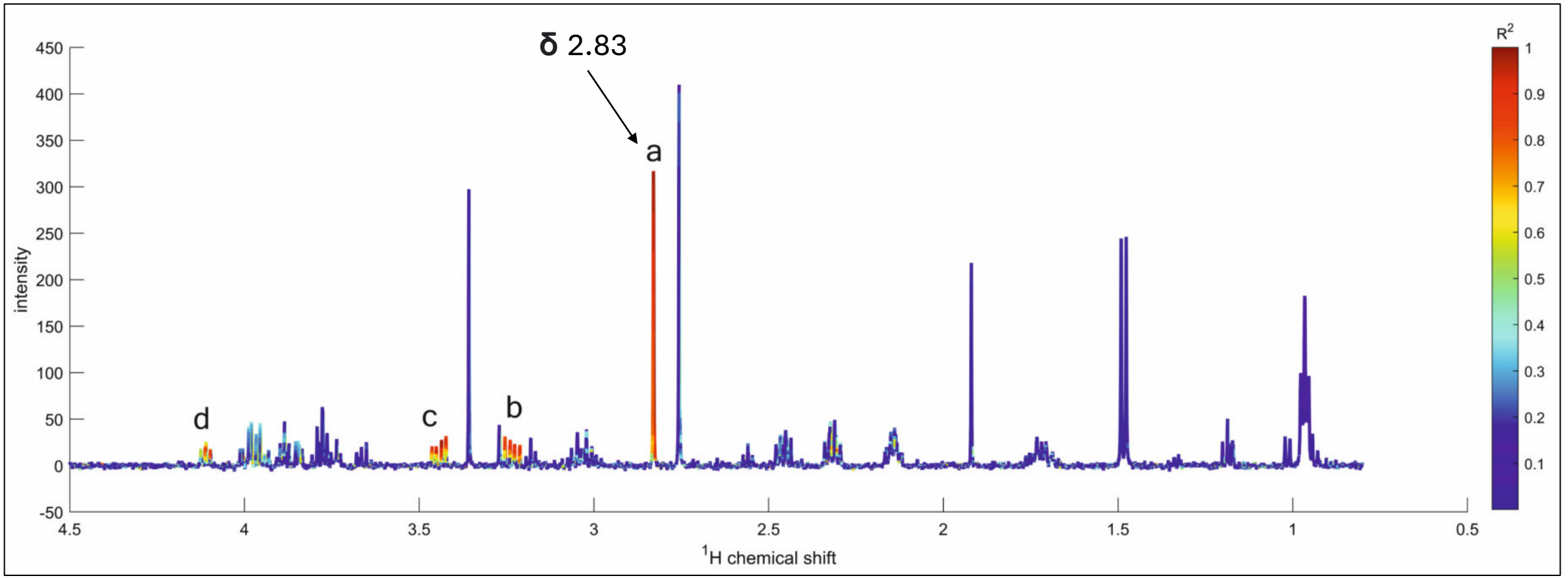
X-axis: the groups summer (red) and winter (green), y-axis: metabolite concentration (ug/bee), black bar: mean

NMR-based compound identification and fractionation



S-methyl-L-Cysteine-S-oxide

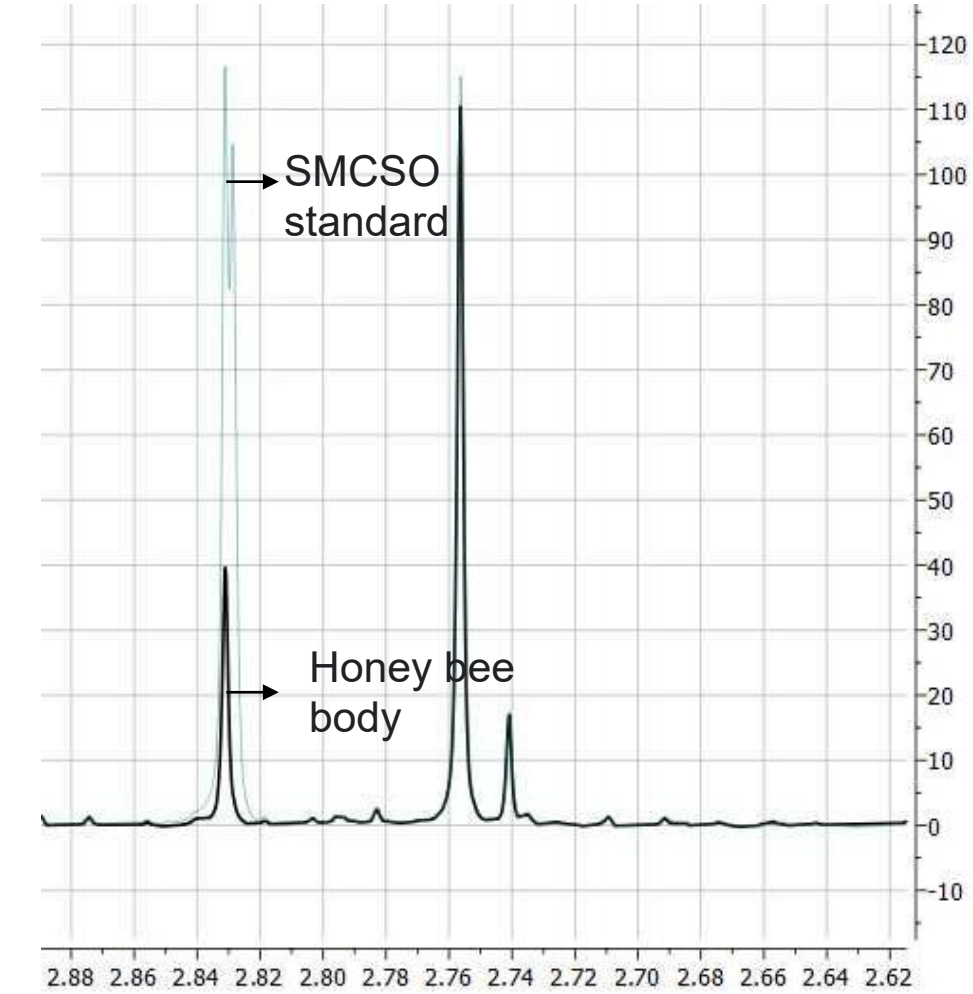
Statistical Total Correlation Spectroscopy (STOCSY)
Helps to find the correlated signals with designated peak



Lee, 2024, Open Biology

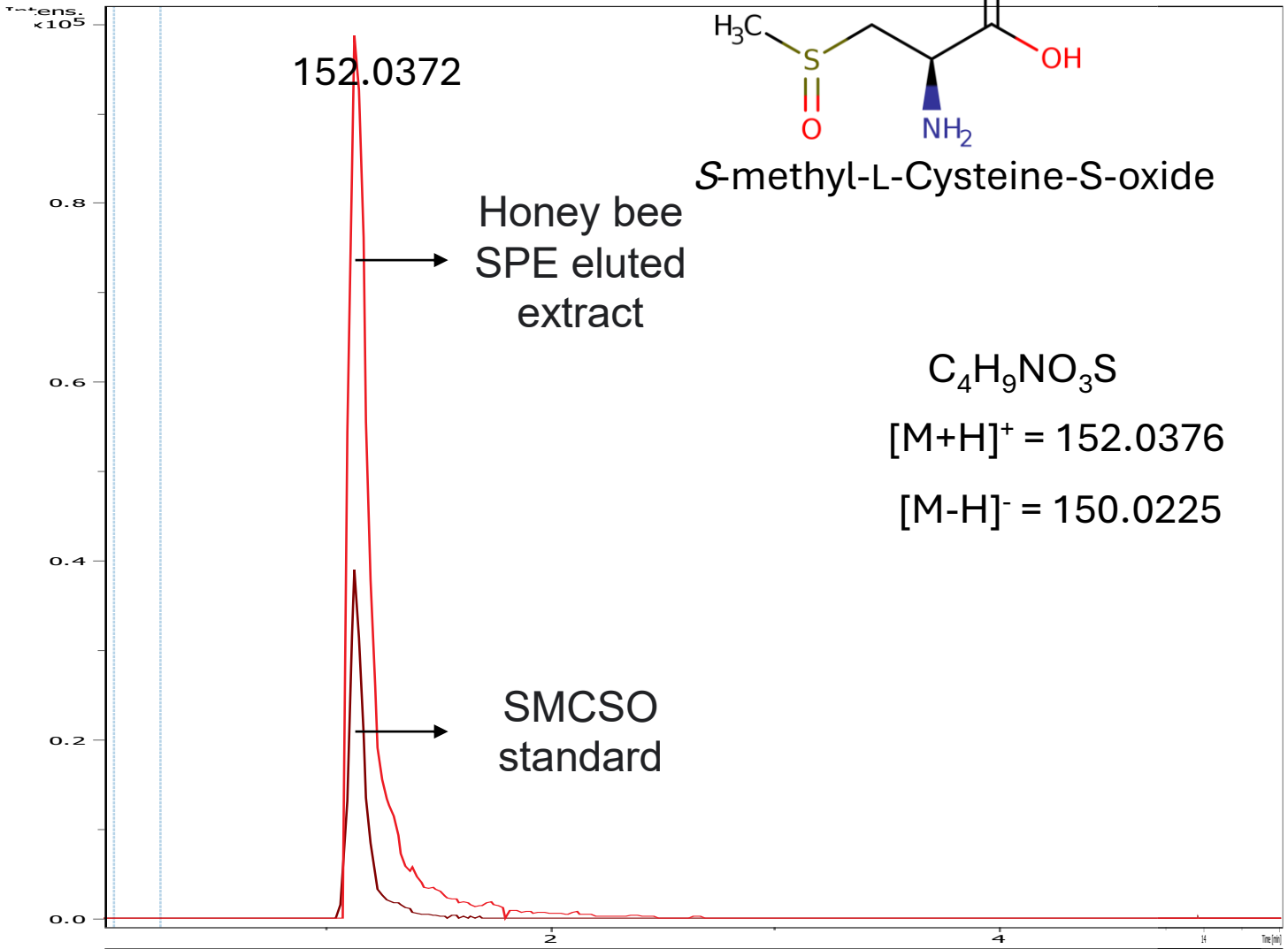
NMR and MS-based compound identification

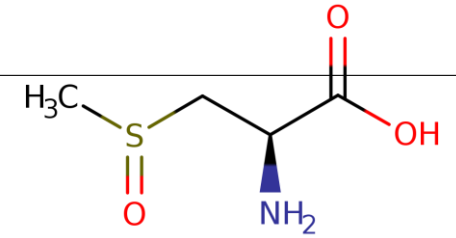
NMR spike



Lee, 2024, Open Biology

LC-MS/MS





S-methyl-L-Cysteine-S-oxide

$C_4H_9NO_3S$

$[M+H]^+ = 152.0376$

$[M-H]^- = 150.0225$

Literature Research

Journal of proteome research

ARTICLE

pubs.acs.org/jpr

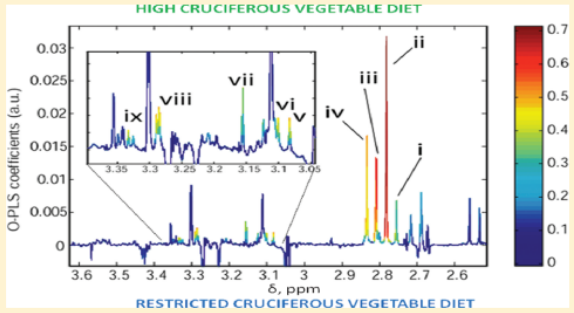
Identification of Human Urinary Biomarkers of Cruciferous Vegetable Consumption by Metabonomic Profiling

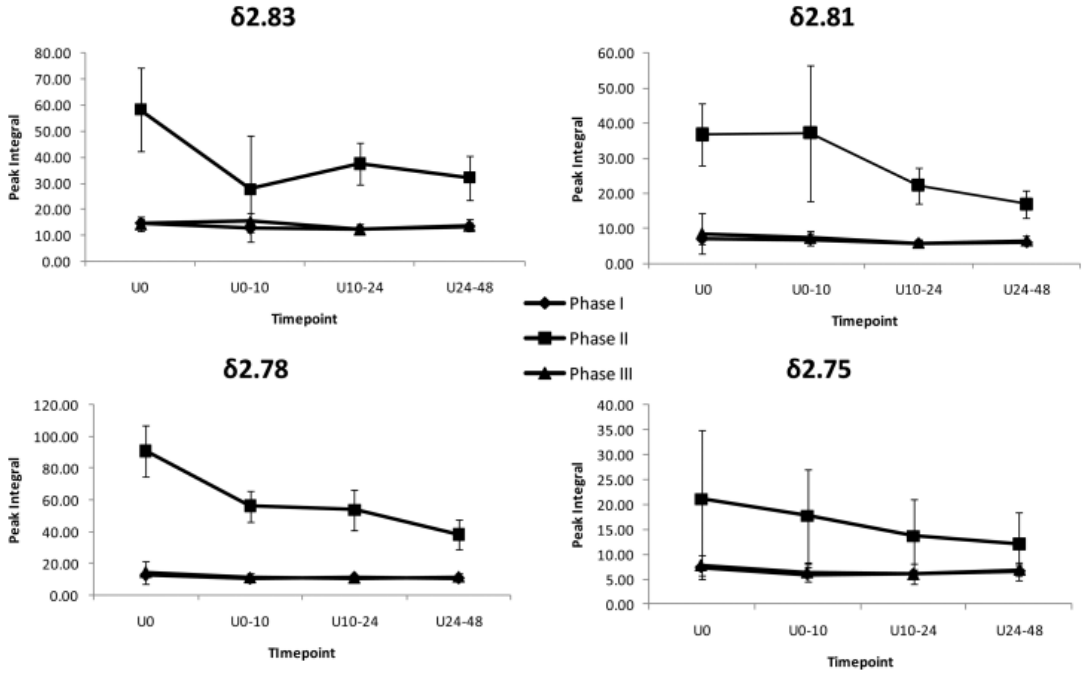
William M. B. Edmands,[†] Olaf P. Beckonert,[†] Cinzia Stella,[†] Alison Campbell,[†] Brian G. Lake,[‡] John C. Lindon,[†] Elaine Holmes,[†] and Nigel J. Gooderham*[,†]

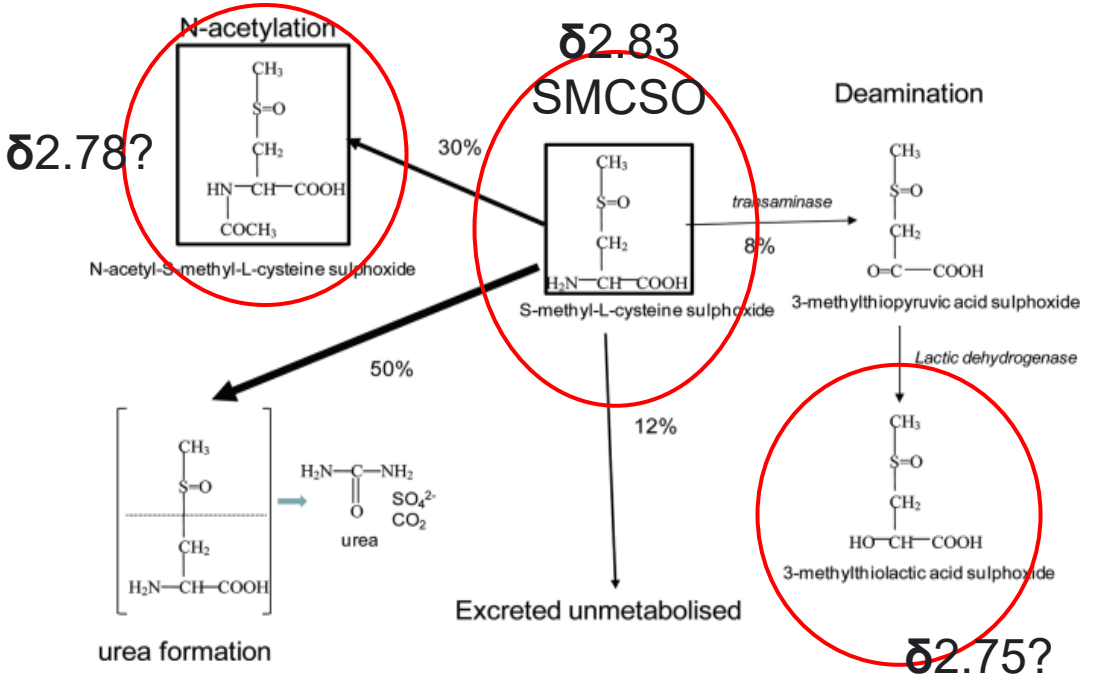
[†]Biomolecular Medicine, Department of Surgery and Cancer, Faculty of Medicine, Imperial College London, South Kensington, London SW7 2AZ, United Kingdom

[‡]Leatherhead Food International, Leatherhead, KT22 7RY, United Kingdom

ABSTRACT: Consumption of cruciferous vegetables (CVs) is inversely correlated to many human diseases including cancer (breast, lung, and bladder), diabetes, and cardiovascular and neurological disease. Presently, there are no readily measurable biomarkers of CV consumption and intake of CVs has relied on dietary recall. Here, biomarkers of CV intake were identified in the urine of 20 healthy Caucasian adult males using ^{1}H NMR spectroscopy with multivariate statistical modeling. The study was separated into three phases of 14 days: a run-in period with restricted CV consumption (phase I); a high CV phase where participants consumed 250 g/day of both broccoli and Brussels sprouts (phase II); a wash-out phase with a return to restricted CV consumption (phase III). Each study participant provided a complete cumulative urine collection over 48 h at the end of each phase; a spot urine (U0), 0−10 h (U0−10), 10−24 h (U10−24), and 24−48 h (U24−48) urine samples. Urine samples obtained after consumption of CVs were differentiated from low CV diet samples by four singlet ^{1}H NMR spectroscopic peaks, one of which was identified as *S*-methyl-L-cysteine sulfoxide (SMCSO) and the three other peaks were tentatively identified as other metabolites structurally related to SMCSO. These stable urinary biomarkers of CV consumption will facilitate future assessment of CVs in nutritional population screening and dietary intervention

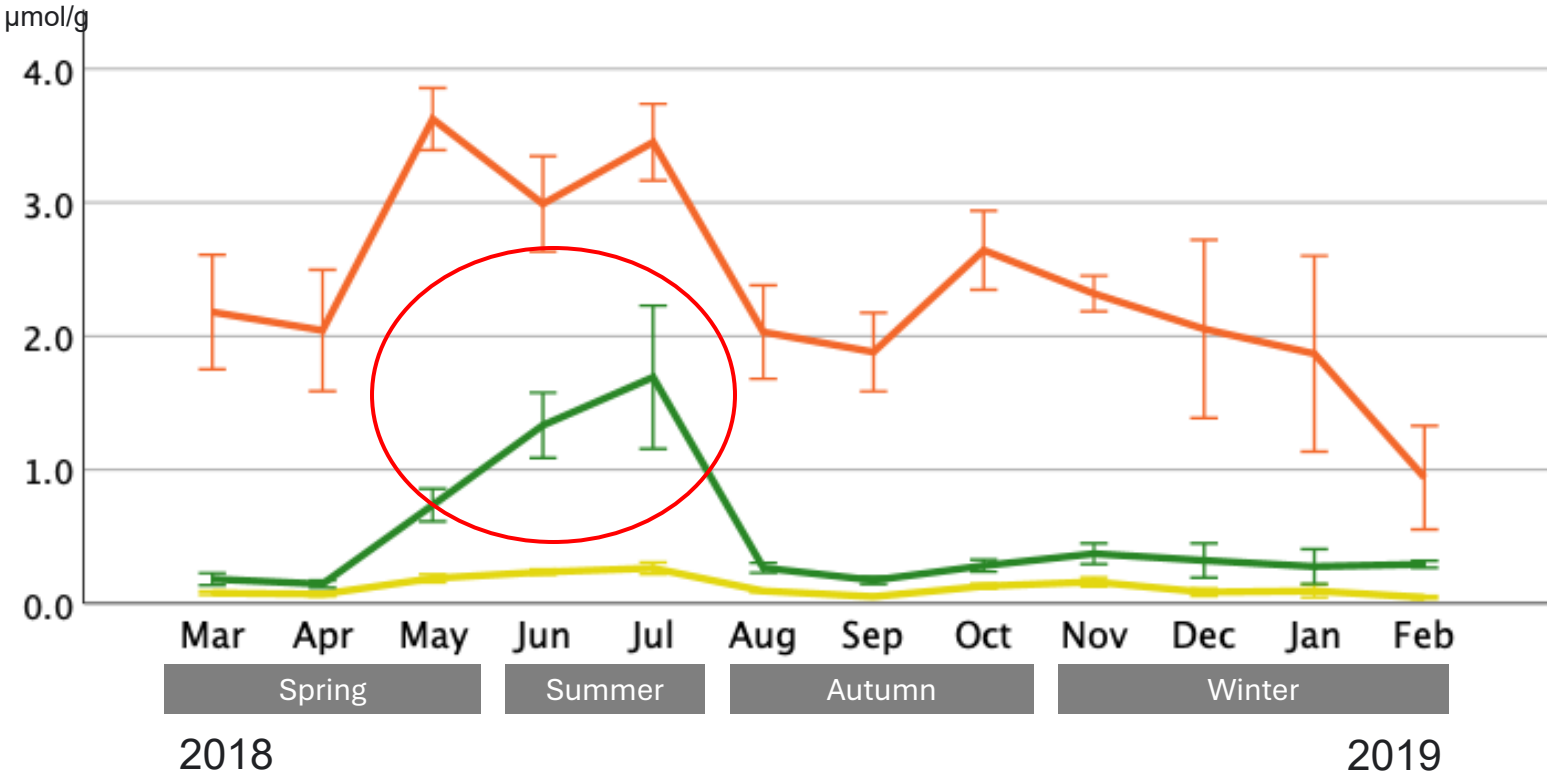


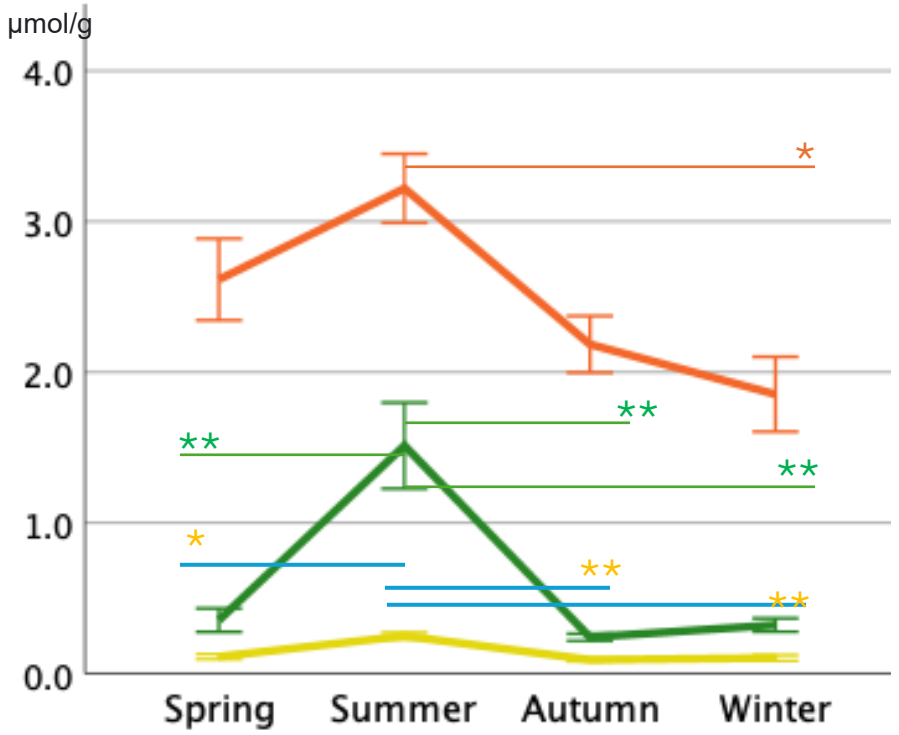




Experiment 2: Monthly variations in honey bee bodies

- 3Me-TLASO
- NAc-SMCSO
- SMCSO



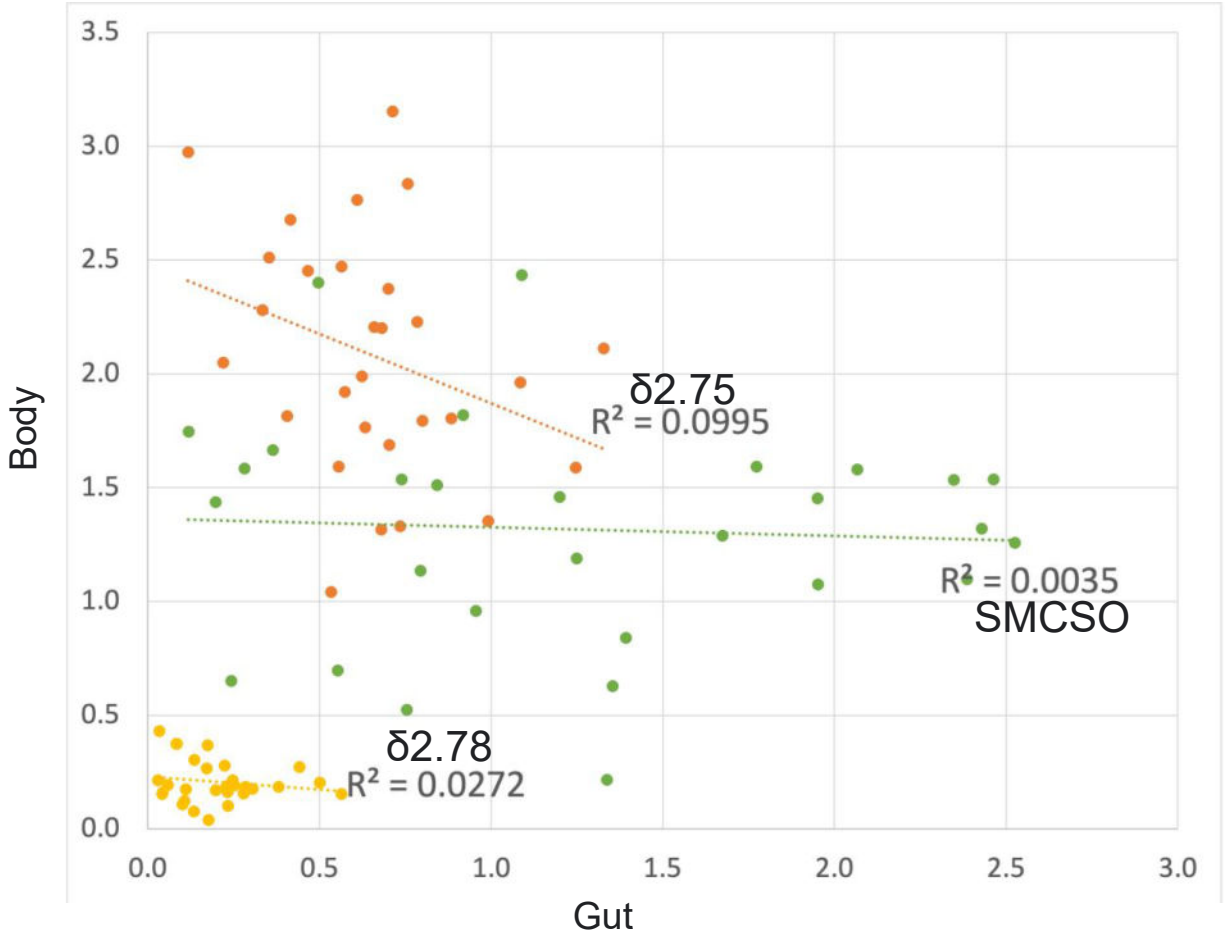


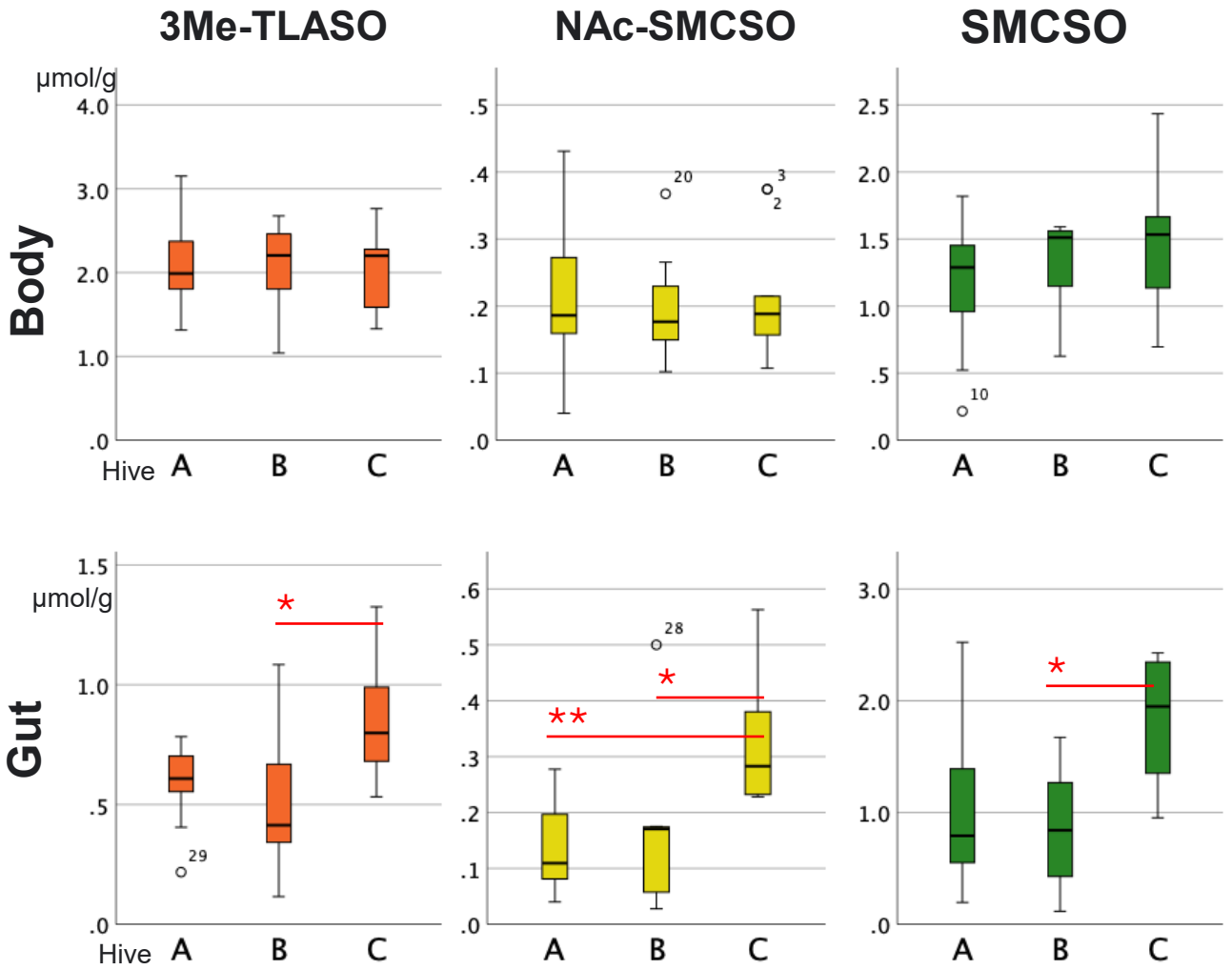
ANOVA, Bonferroni FDR
$p < 0.005$, ** $p < 0.001$
+/- SEM

Lee, 2024, Open Biology

Experiment 3: Body and gut distribution

ANOVA, Bonferroni FDR
* $p < 0.05$, ** $p < 0.005$

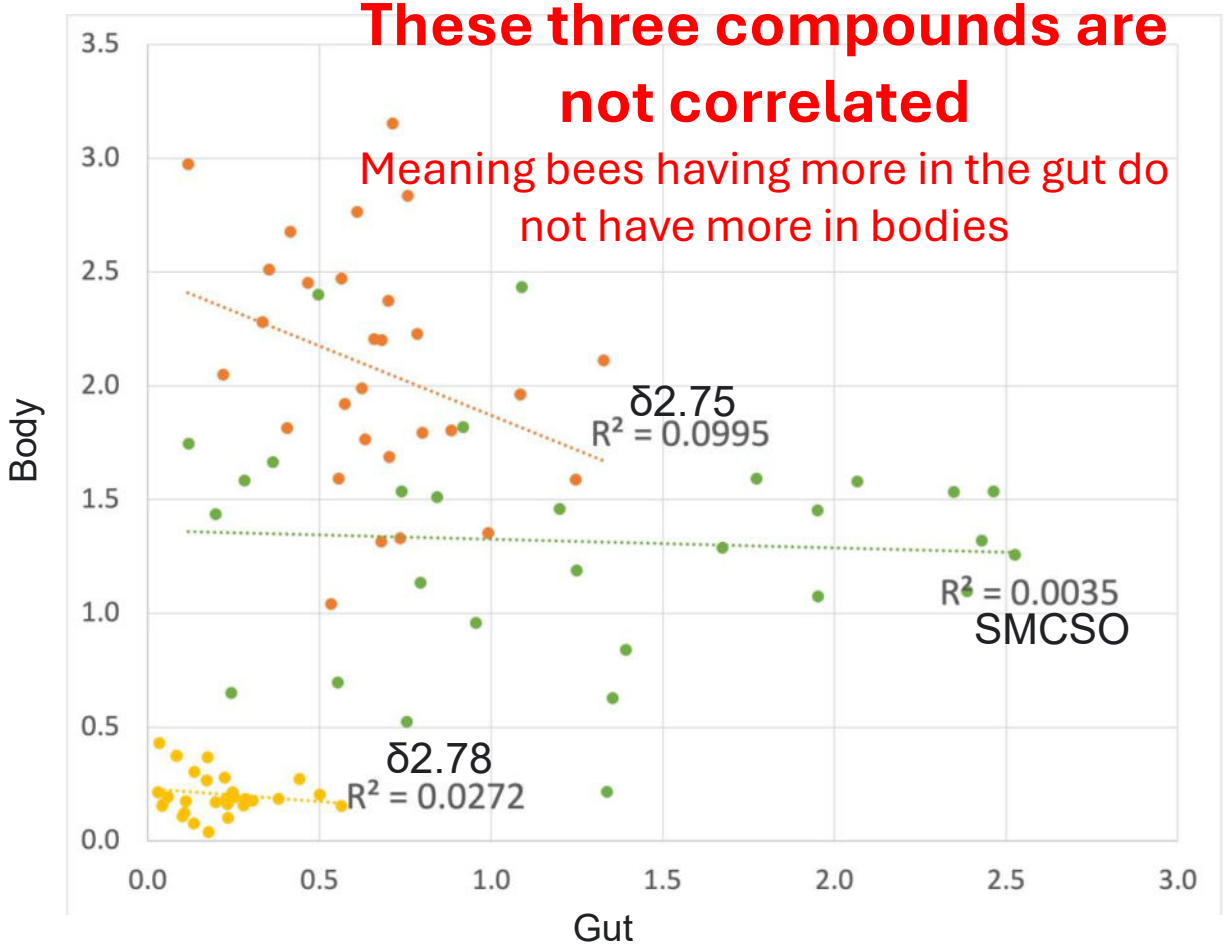


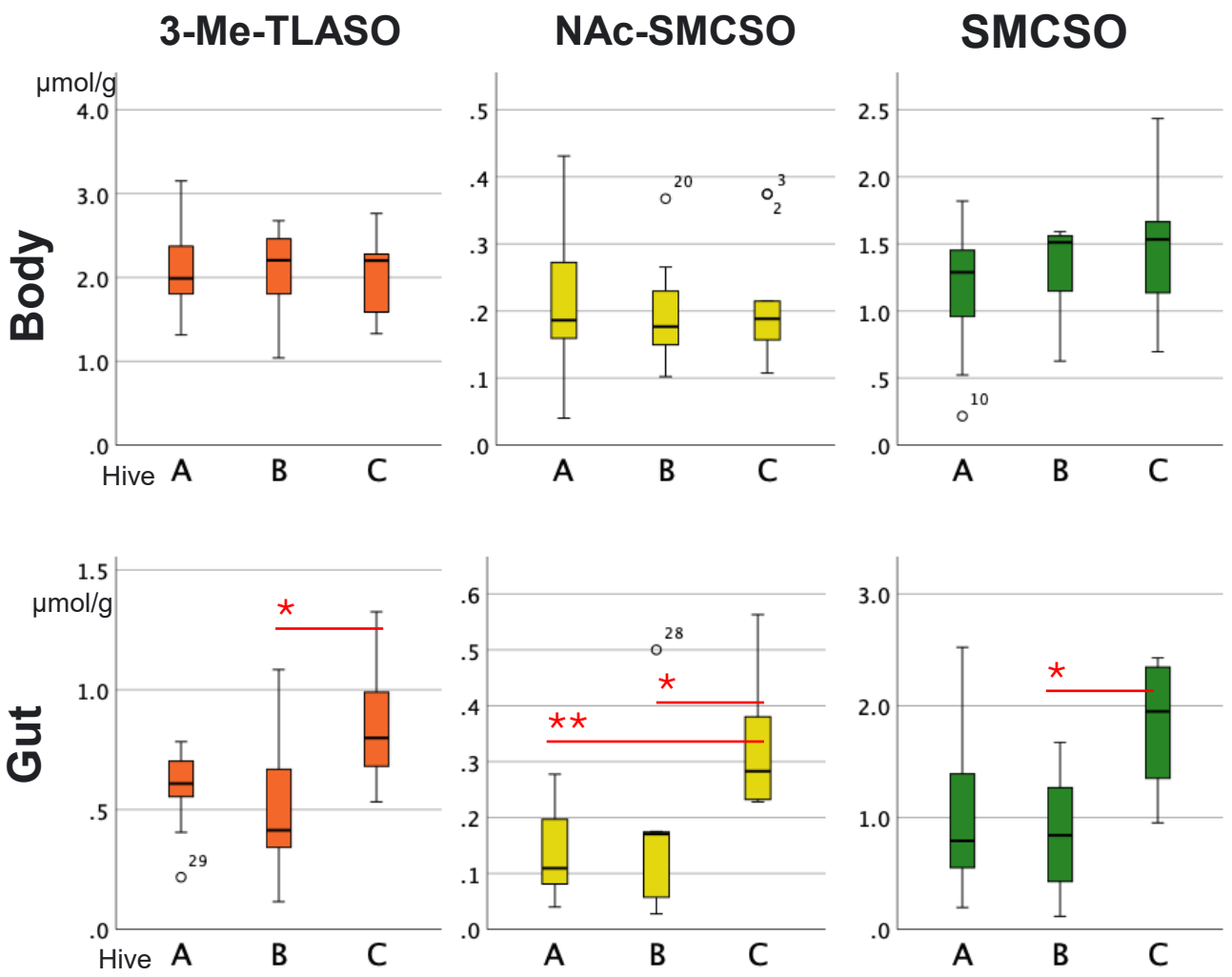


Lee, 2024, Open Biology

Experiment 3: Body and gut distribution

ANOVA, Bonferroni FDR
* $p < 0.05$, ** $p < 0.005$

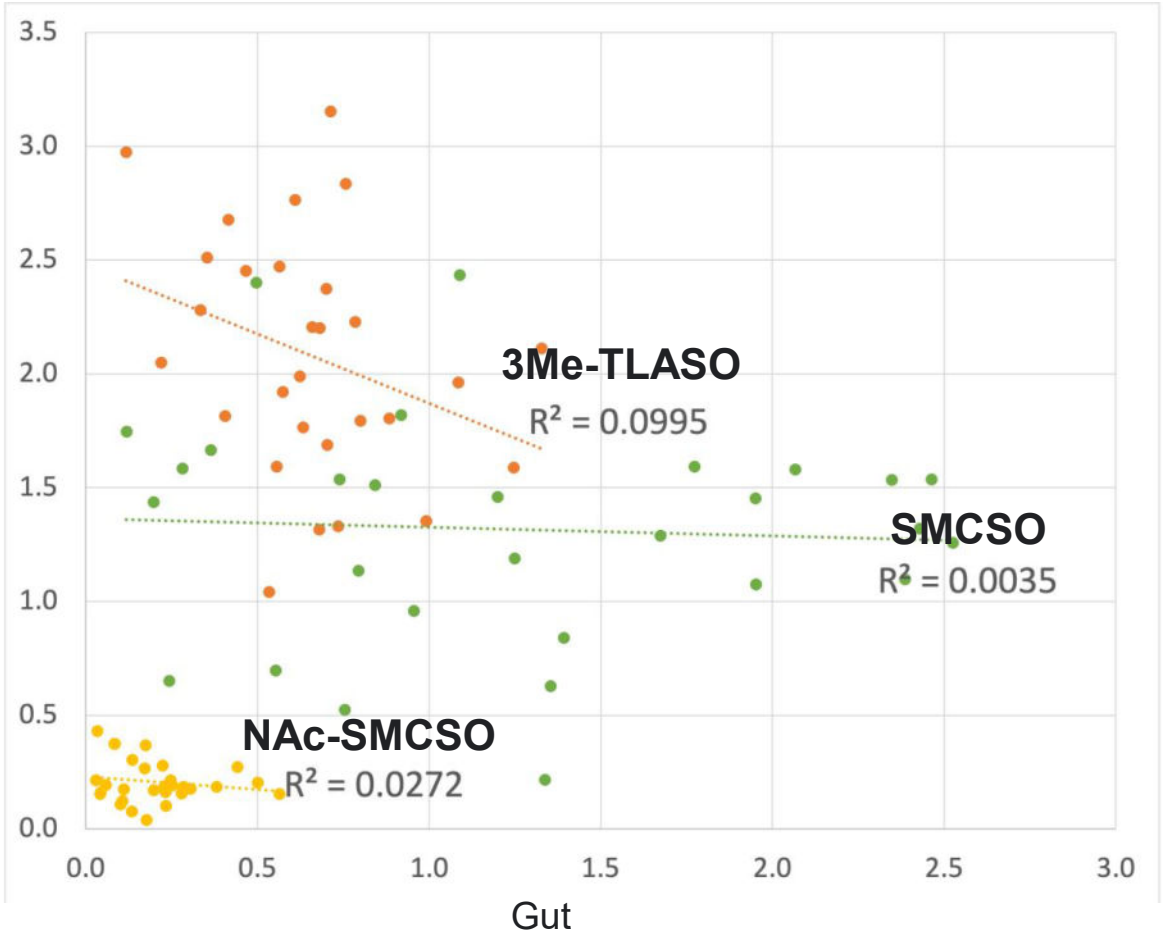


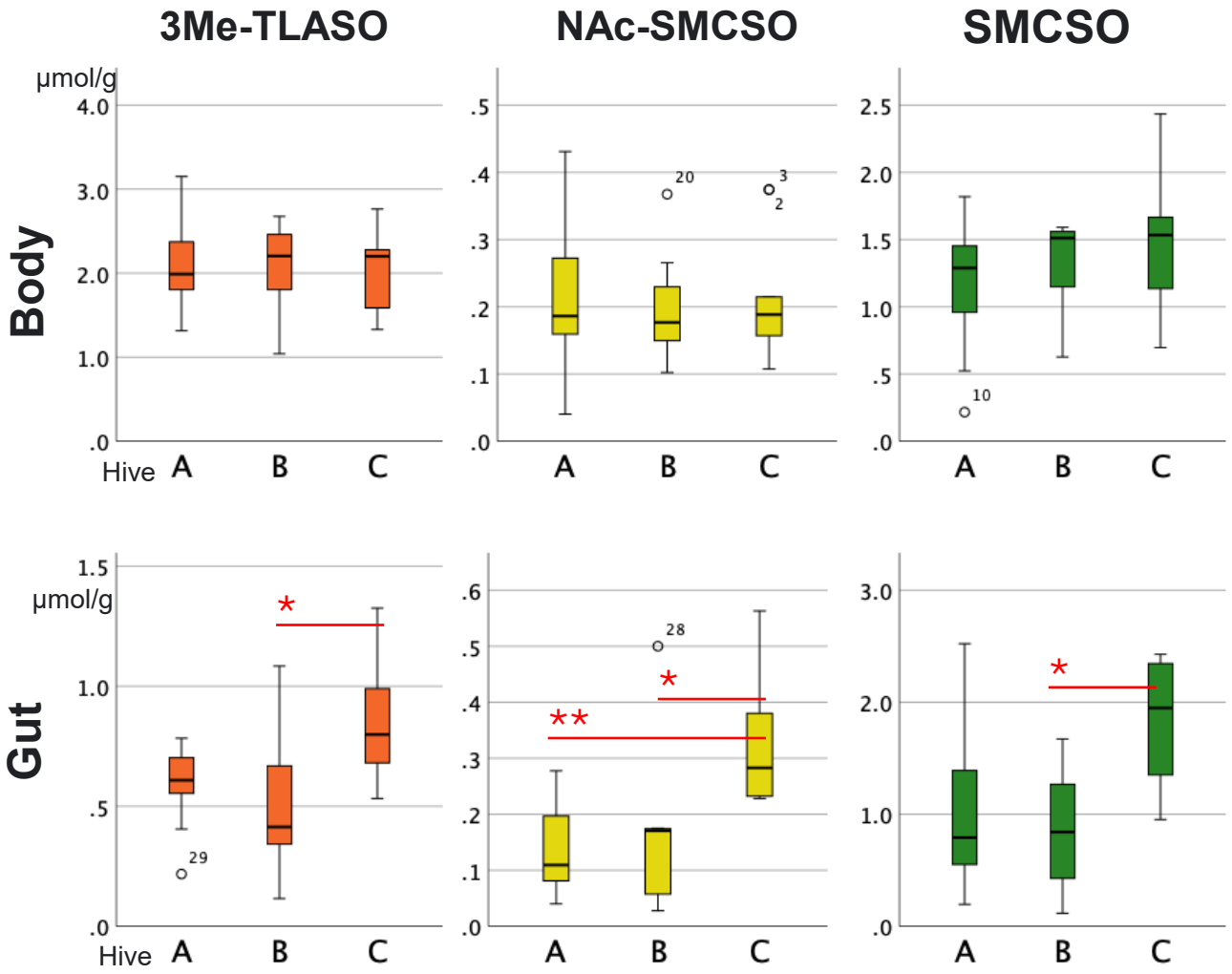


Lee, 2024, Open Biology

Experiment 3: Body and gut distribution

ANOVA, Bonferroni FDR
* $p < 0.05$, ** $p < 0.005$

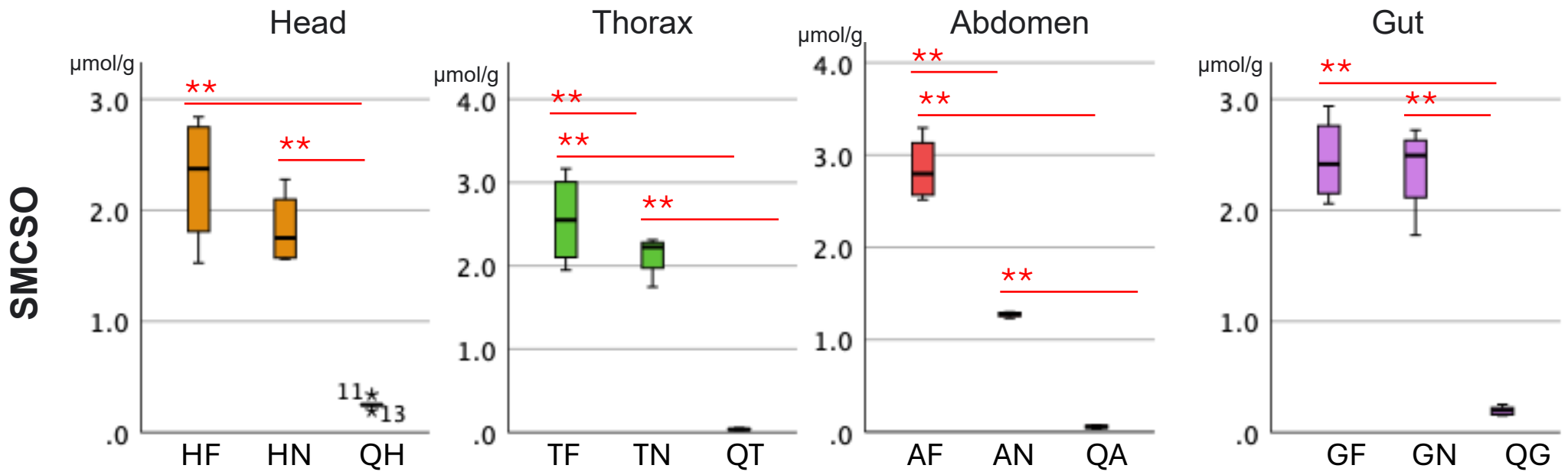




Lee, 2024, Open Biology

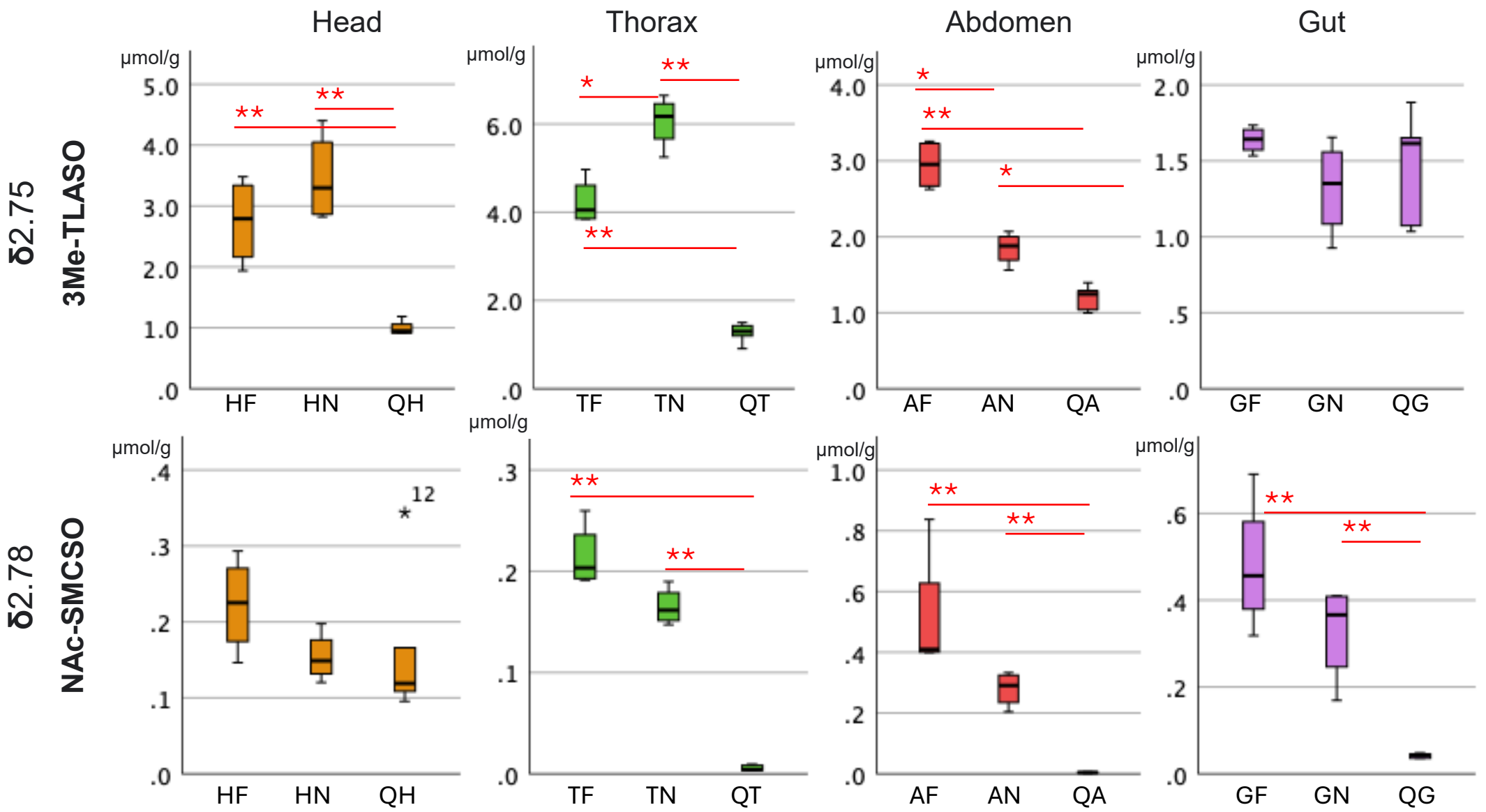
But bees having more SMCSO in the gut have also more metabolites

Experiment 4: Body parts and castes distribution



ANOVA,
Bonferroni FDR
* $p < 0.01$
** $p < 0.001$

Experiment 4: Body parts and castes distribution

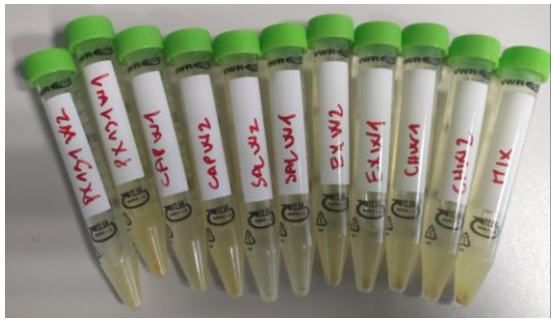


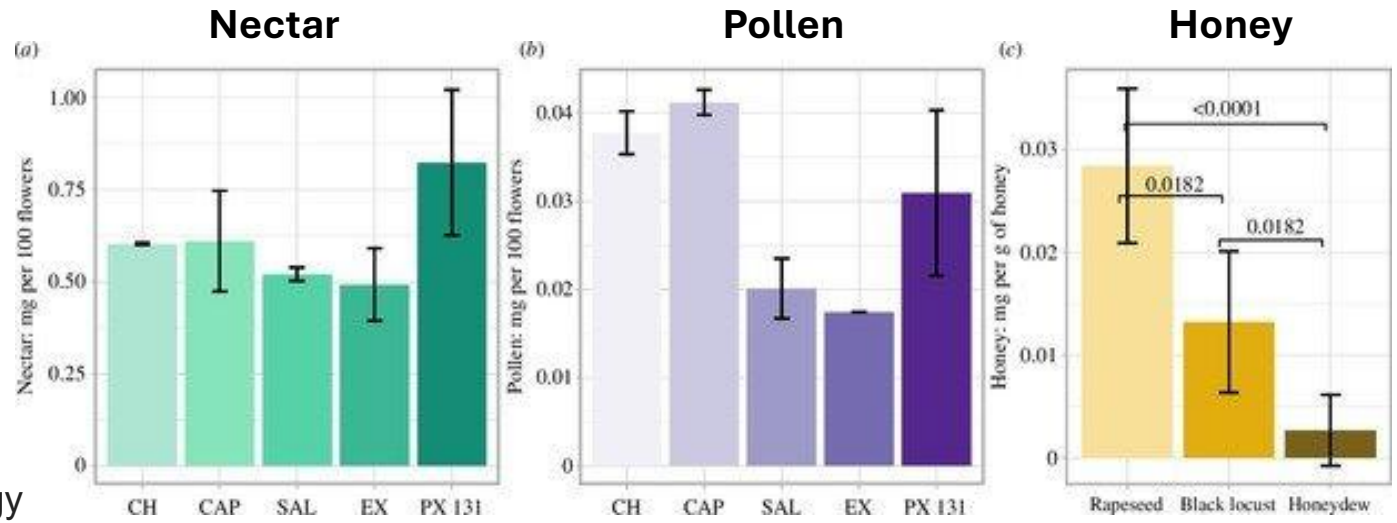
ANOVA,
Bonferroni FDR
* $p < 0.01$
** $p < 0.001$

Experiment 5: Origin of SMCSO

SMCSO originates from rapeseed

- Hypothesis: **SMCSO originates from rapeseed**. The objective was to quantify SMCSO content in pollen and nectar of flowers rapeseed varieties and its content in varietal honeys
- Rapeseed flowers (n = 100) from 4 varieties collected at same experimental field
- Results expressed per 100 flowers



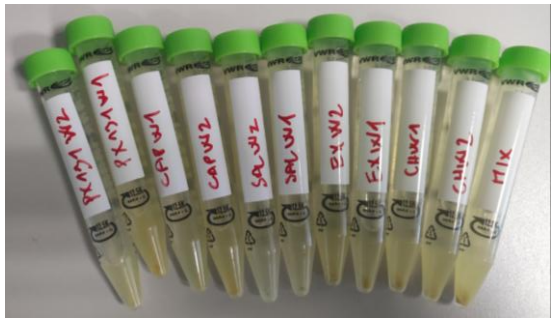


Lee, 2024, Open Biology

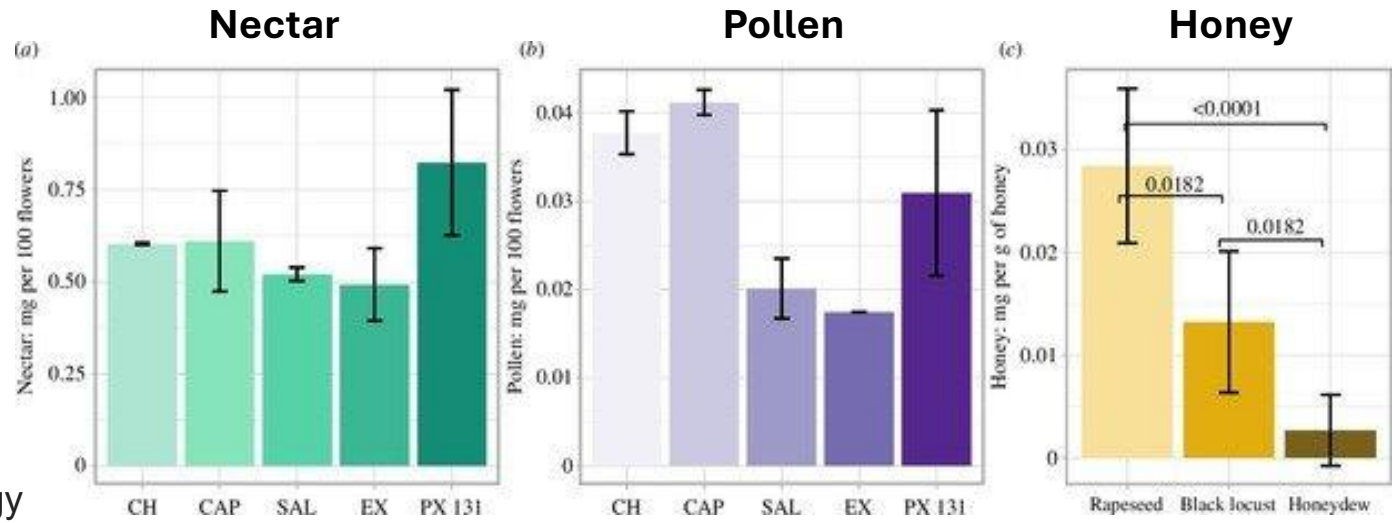
Experiment 5: Origin of SMCSO

SMCSO originates from rapeseed

- Hypothesis: **SMCSO originates from rapeseed**. The objective was to quantify SMCSO content in pollen and nectar of flowers rapeseed varieties and its content in varietal honeys
- Rapeseed flowers (n = 100) from 4 varieties collected at same experimental field
- Results expressed per 100 flowers



Main source of SMCSO is nectar and it is present in rapeseed honey



Lee, 2024, Open Biology

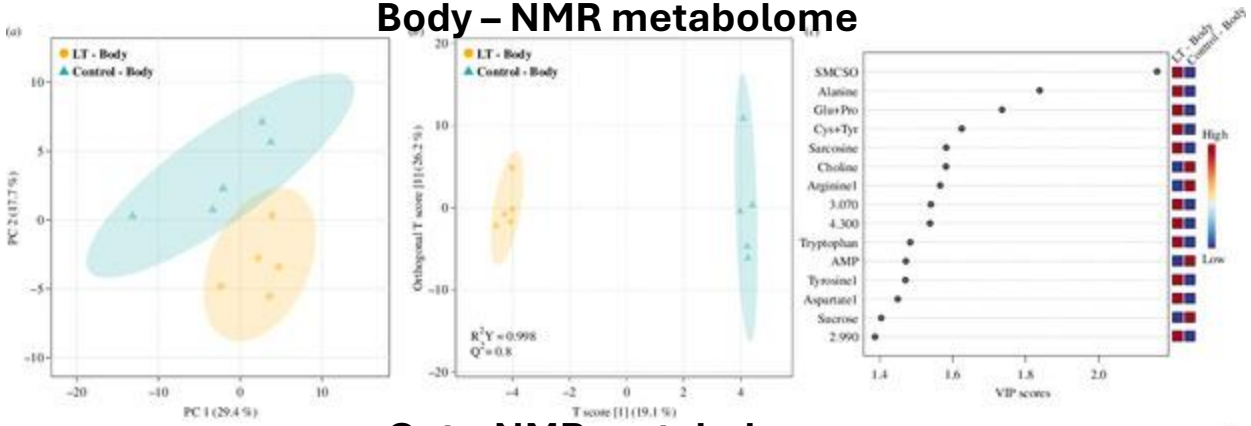
Experiment 6: Toxicity, biological activity of SMCSO

SMCSO affects bee health in a negative or positive way

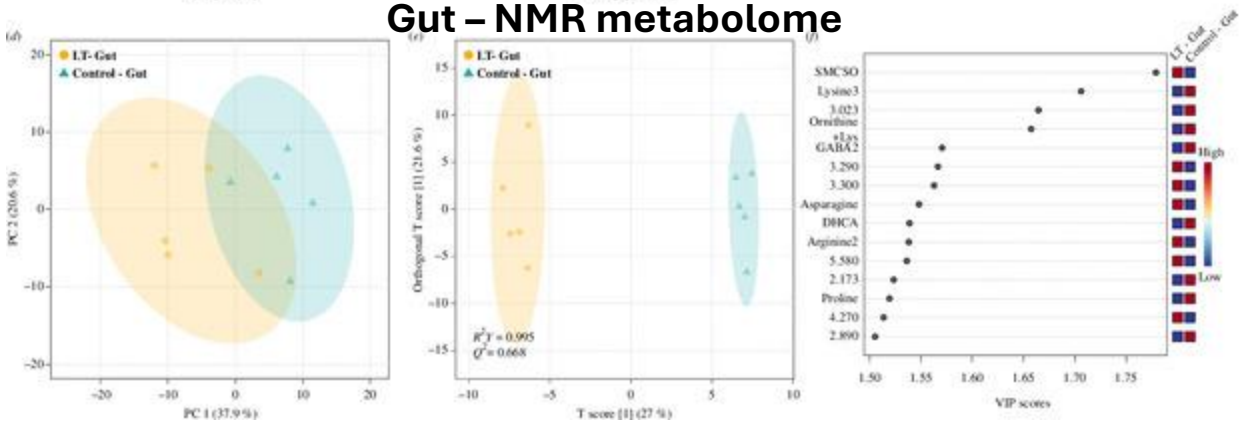
- Hypothesis: **SMCSO shows antioxidant, antimicrobial activity or toxicity and alters metabolism.** The objective was to conduct acute and long-term feeding study in experimental cages, pure SMCSO was administered 1-time or regularly in the feeding solution

50 bees/cage, n=5 pooled samples

Body – NMR metabolome



Gut – NMR metabolome



Lee, 2024, Open Biology

Experiment 6: Toxicity, biological activity of SMCSO

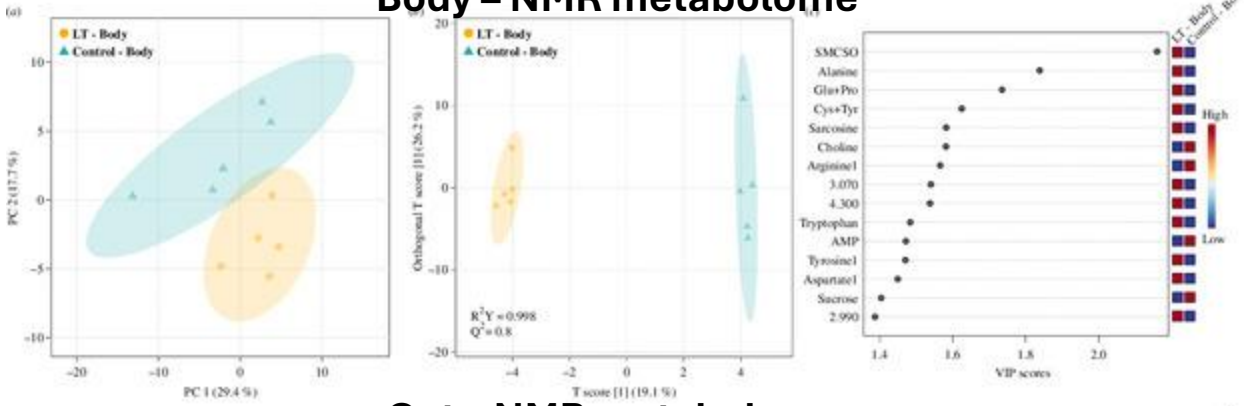
SMCSO affects bee health in a negative or positive way

- Hypothesis: **SMCSO shows antioxidant, antimicrobial activity or toxicity and alters metabolism.** The objective was to conduct acute and long-term feeding study in experimental cages, pure SMCSO was administered 1-time or regularly in the feeding solution

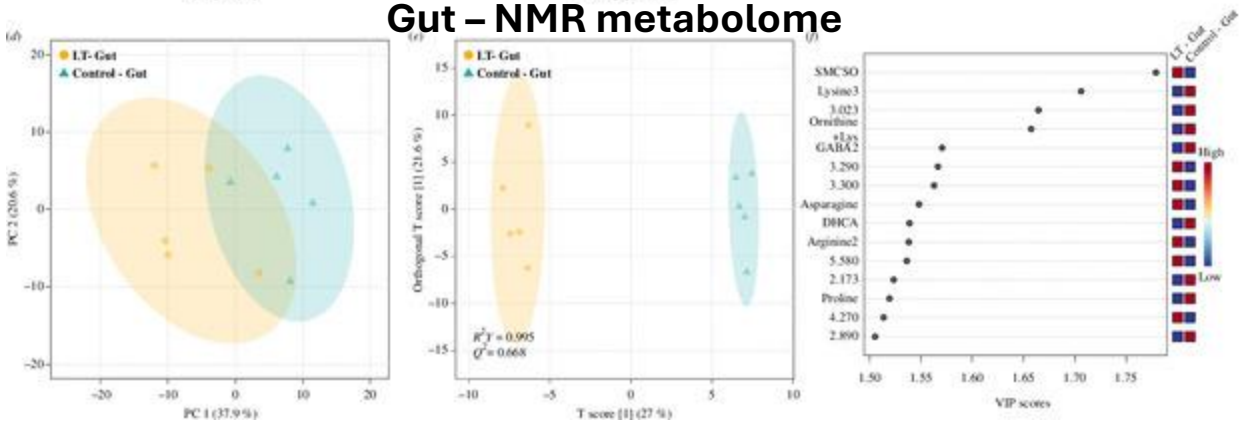
SMCSO administration has an effect on body amino acid profiles

In the gut it affects mainly unknown metabolites

Body – NMR metabolome



Gut – NMR metabolome



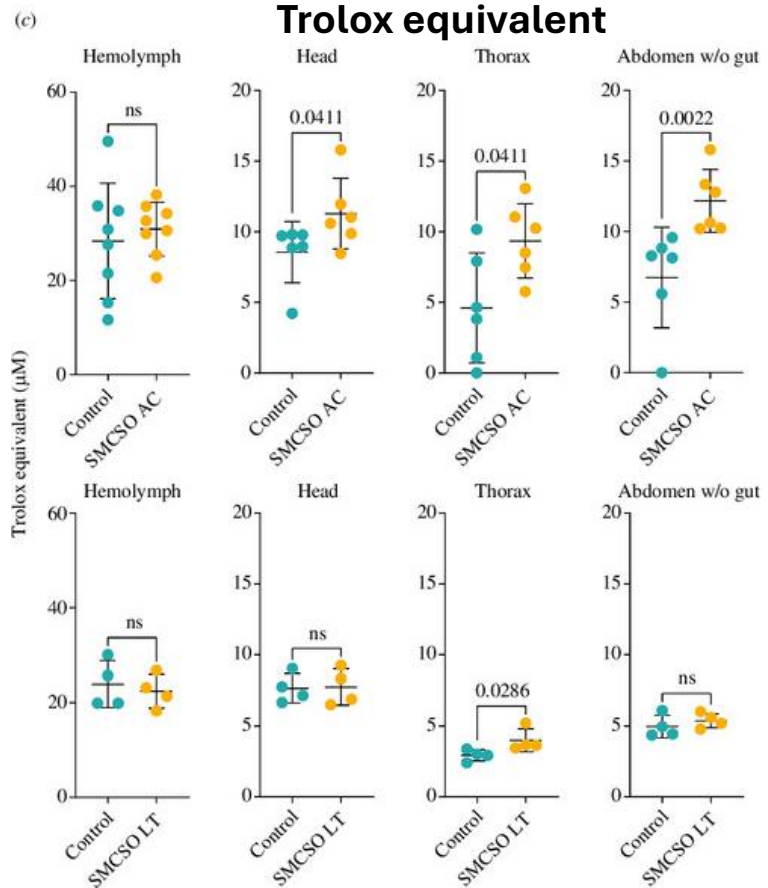
Lee, 2024, Open Biology

Experiment 6: Toxicity, biological activity of SMCSO

SMCSO affects bee health in a negative or positive way

- Hypothesis: **SMCSO shows antioxidant, antimicrobial activity or toxicity and alters metabolism.** The objective was to conduct acute and long-term feeding study in experimental cages, pure SMCSO was administered 1-time or regularly in the feeding solution

SMCSO affects antioxidant activit, mainly in thorax (both in acute and long-term feeding)



AC, acute feeding, 1 mg/ml one-time to 50 bees, LT, long-term feeding, 1 mg/ml ad libitum to 50 bees, n=7 samples measured

Lee, 2024, Open Biology

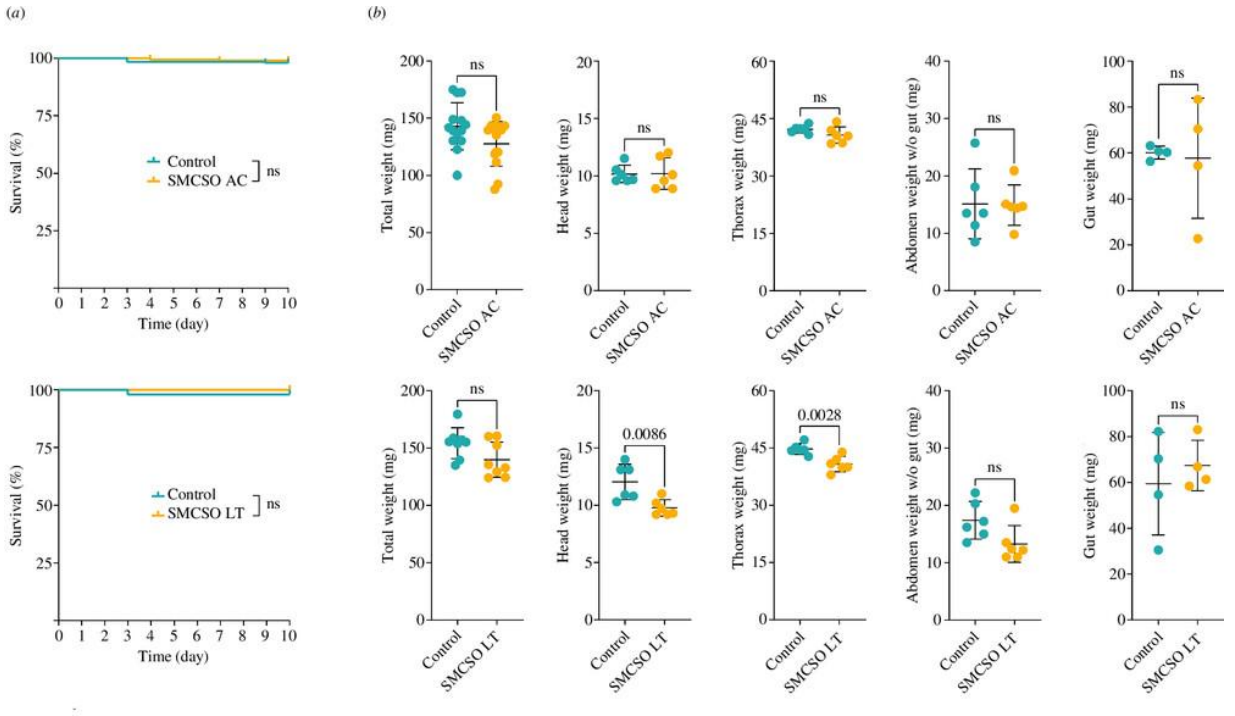
Experiment 6: Toxicity, biological activity of SMCSO

SMCSO affects bee health in a negative or positive way

- Hypothesis: **SMCSO shows antioxidant, antimicrobial activity or toxicity and alters metabolism.** The objective was to conduct acute and long-term feeding study in experimental cages, pure SMCSO was administered 1-time or regularly in the feeding solution

SMCSO did not show marked toxicity at 1 mg/ml during acute or long-term feeding, but bees tended to have lower weight during acute exposure

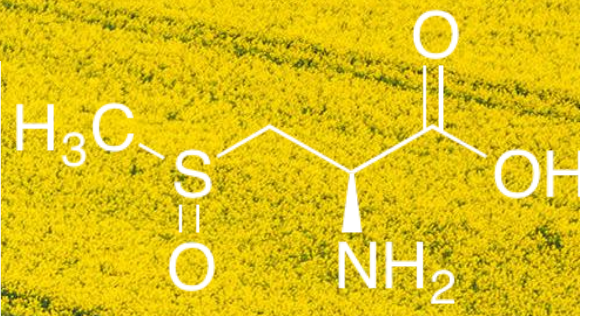
Weight of selected body parts



AC, acute feeding, 1 mg/ml one-time to 50 bees
LT, long-term feeding, 1 mg/ml ad libitum to 50 bees

Lee, 2024, Open Biology

Conclusion

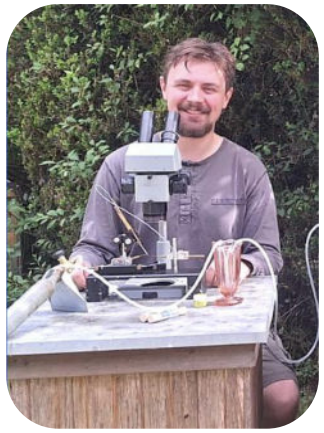


- SMCSO is **a hidden layer in bee pollination biology** (Bellec et al, Plant Cell Environ, 2025)
- Not toxic at tested levels, but initial results suggest alterations of body amino acid metabolism
- Body-part distribution revealed deposition mainly is thorax(muscles) in foragers
- Seasonal peak in Jun-July – post-rapeseed bloom.
- Potentially important for colony resilience?
- **NMR, NMR, NMR!!!**

Acknowledgements

AGROMICS
reasearch
team

Czech
University
of Life Sciences
Prague



VÝZKUMNÝ ÚSTAV
VČELAŘSKÝ

MUNI

ÚOCHB AV ČR
IOCB PRAGUE

Special Thanks to

A. Mascellani Bergo

Dr. Radek Pohl

This research was supported by the Ministry of Agriculture of the Czech Republic (QK21010088) and by the European Regional Development Fund-Project No. CZ.02.1.01/0.0/0.0/16_019/0000845.