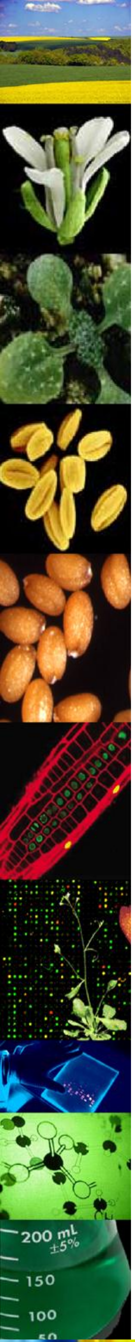
# Saclay Plant Sciences LabEx
# Kick-Off meeting

## **Metabolic interactions and fluxes**

### **Towards a quantitative and qualitative improvement of plant biomass**

October 13, 2011




***Reminder of long-term objectives within the 2d axis :***

**Axe 2: Plants as factories : improving plant quality for food, feed, health and industry**

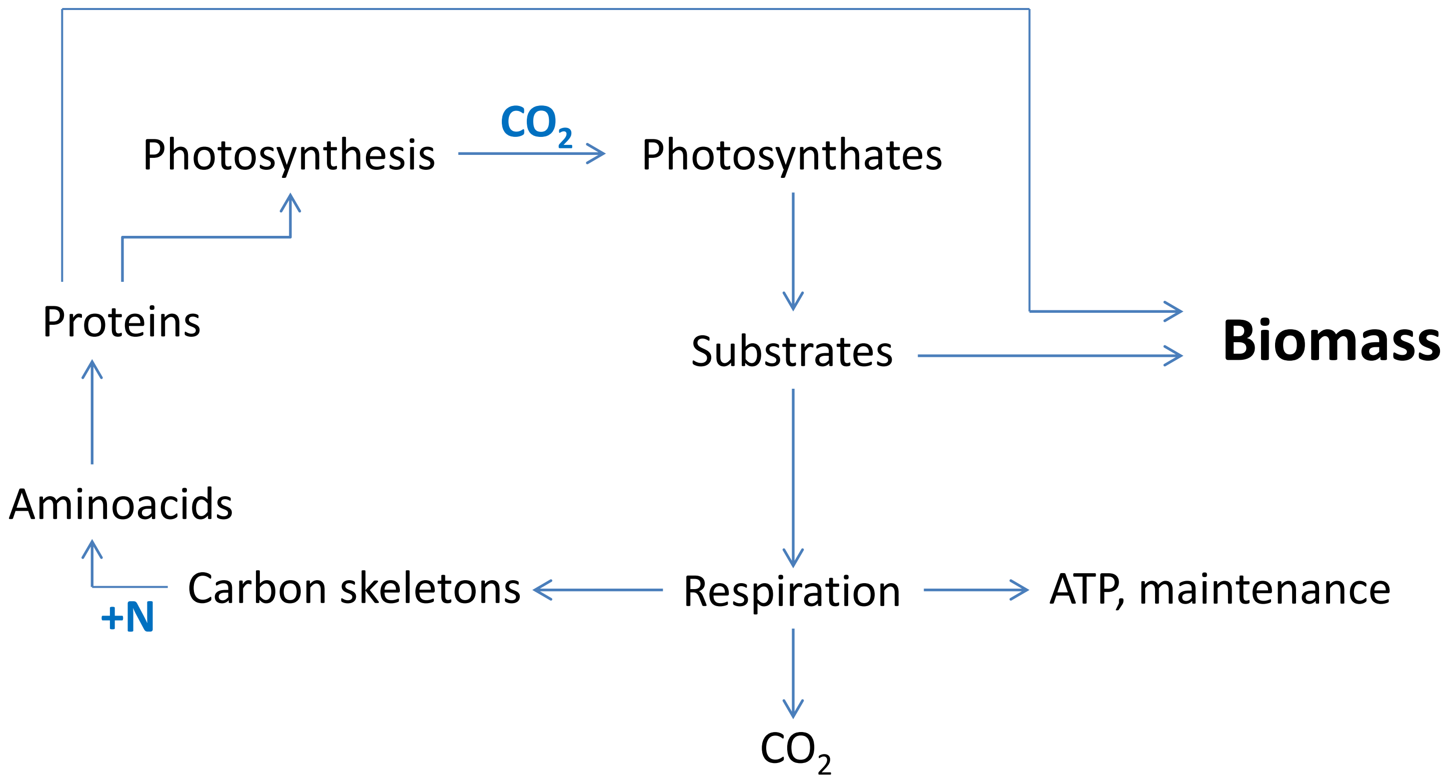
- Structure and regulation of metabolic pathways (biosynthesis, transport, and storage)

- Biosynthesis of biomass adapted to energy production (e.g. ligno-cellulose, oil, sugars)

- Specific molecules for green chemistry (e.g. ligno-cellulose, oil, lipids, proteins, sugars)

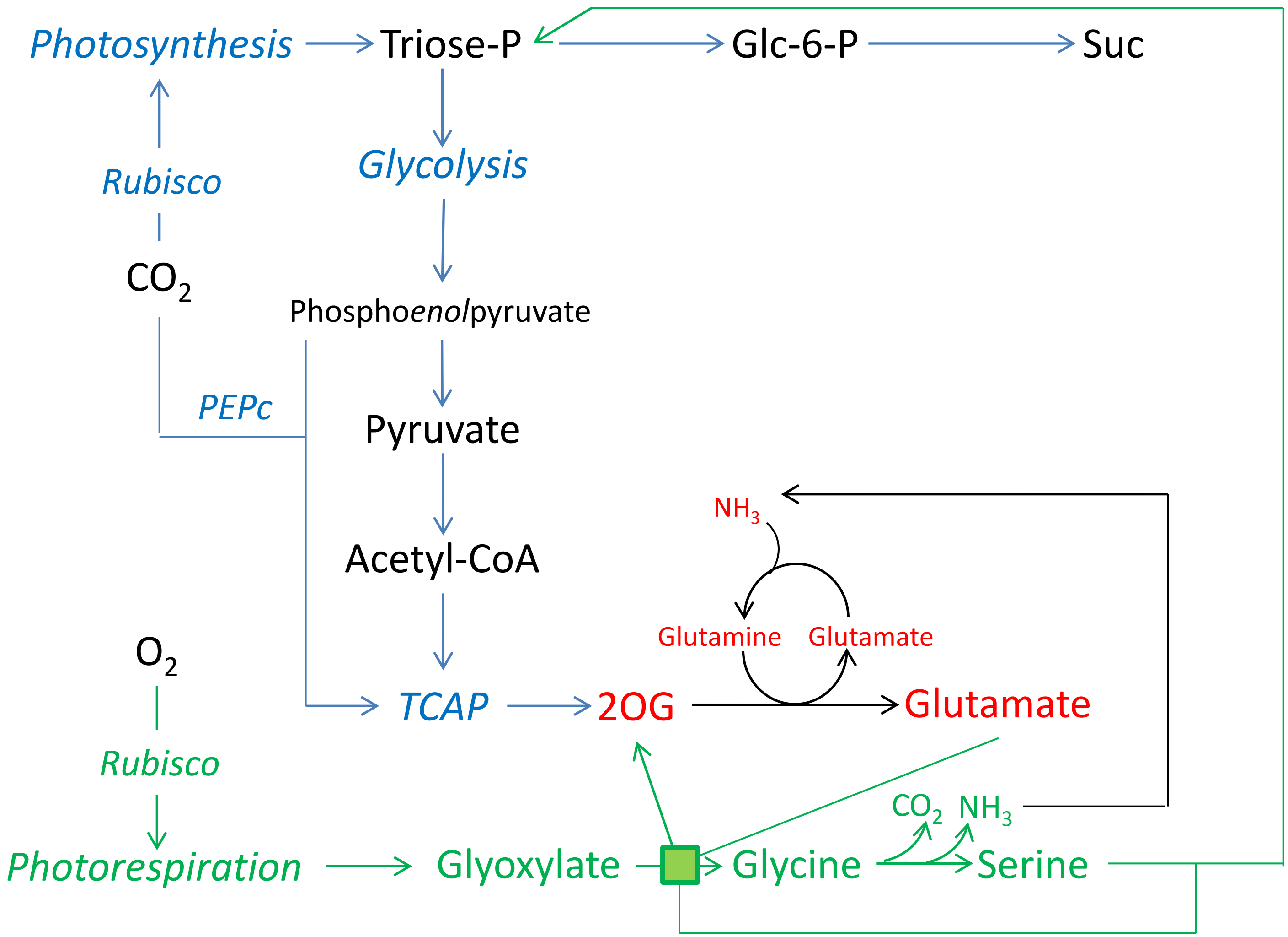
- Production of secondary metabolites for nutrition and health

## *Overall justification:*

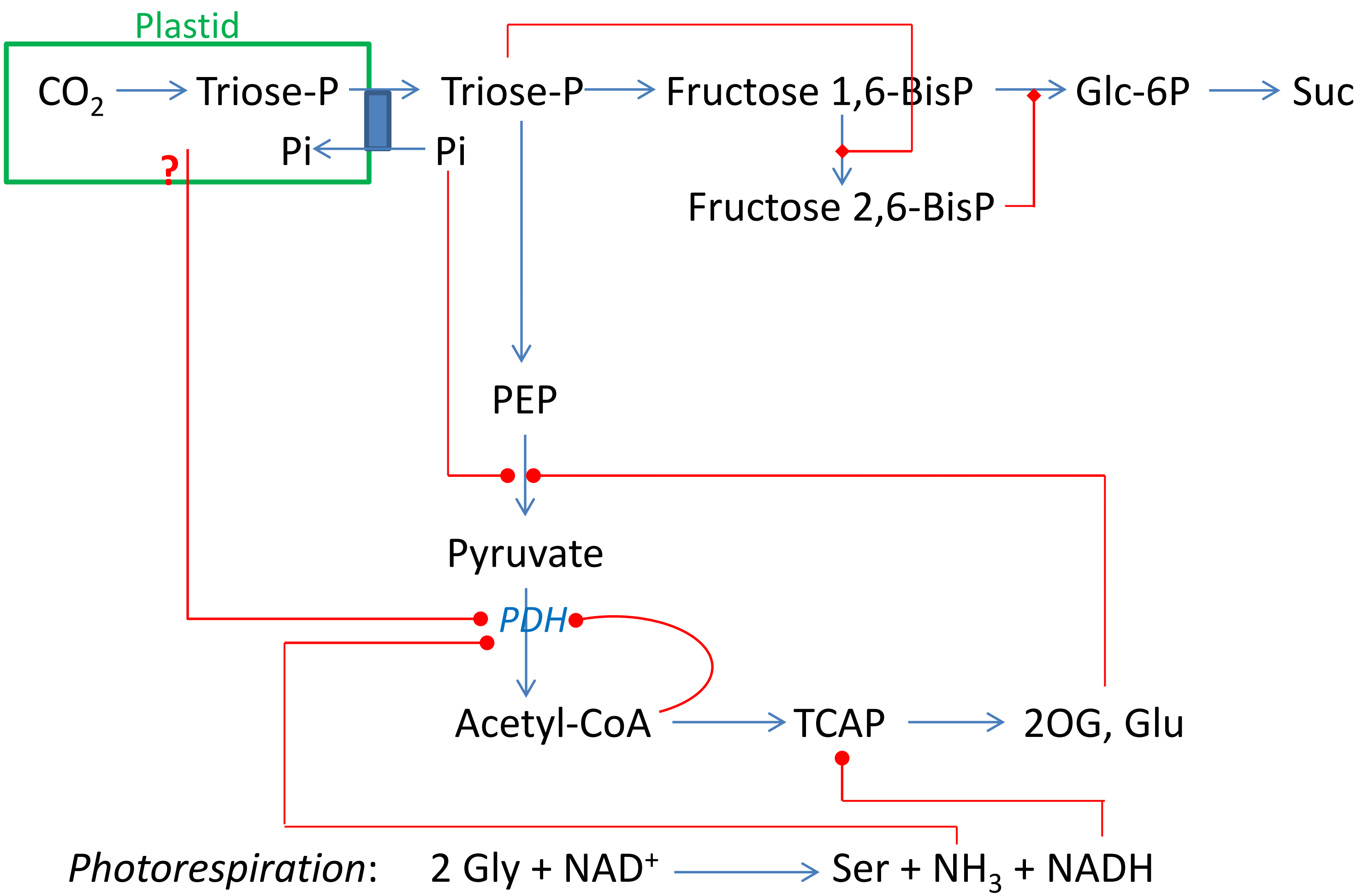



*A very good understanding of **metabolic interactions** and **flux patterns** is required to manipulate carbon allocation to biomass*

# Interactions between key metabolic pathways (leaves)



# What do we know (<u>simplified</u> summary)?



***In addition***

Light/Dark

- Day respiratory $CO_2$ evolution < Night $CO_2$ evolution (Atkin 2000)
- There is little transcriptional or translational control of respiratory activities (Tissue and coworkers, etc.)

$O_2/CO_2$

- On a steady-state basis, 2OG/Glu cycle in photorespiration: no expected influence of photorespiration on day respiration (Buckley and Adams 2011)

but

- Under photorespiratory conditions, there is an increase of nitrate assimilation (Rachmilevitch et al 2003).

## *Important issues*

Is respiration influenced indeed by photorespiration ?

Is nitrogen assimilation into Glu influenced indeed by photorespiration?

How to reconcile respiration inhibition and N assimilation?

What is the importance of reserve recycling?

Are other metabolisms implicated in interactions (e.g. $C_1$-metabolism)?

Are there other pathways involved in providing respiratory intermediates?

What about post-translational light/dark control (phosphorylation, acetylation)?

***Many of these questions can be answered with flux-analyses.***

## *What is the general principle of flux analyses?*

Modern flux analyses include (at least) 3 things:

- Physiological flux measurements: A, $R_n$, ...
- Near-natural abundance isotopics: instantaneous and integrated C sources
- Labeling and tracing: pulse/chase or steady-state iso-distributions

This requires:

- Gas exchange systems, enzymatic activity measurements, etc.
- Isotope ratio mass spectrometry coupled to either GC or gas-exchange
- Enriched substrates and NMR-analysis or MS analyses

## *NMR analysis*

Allows $^{13}$C, $^{15}$N, deuterium, $^{33}$S detection ...

... in <u>each atomic position</u> of interest

Allows detection of <u>double</u> labeling and labeling of <u>neighbouring</u> positions

A full spectrum of <u>all major metabolites</u> in a single analysis

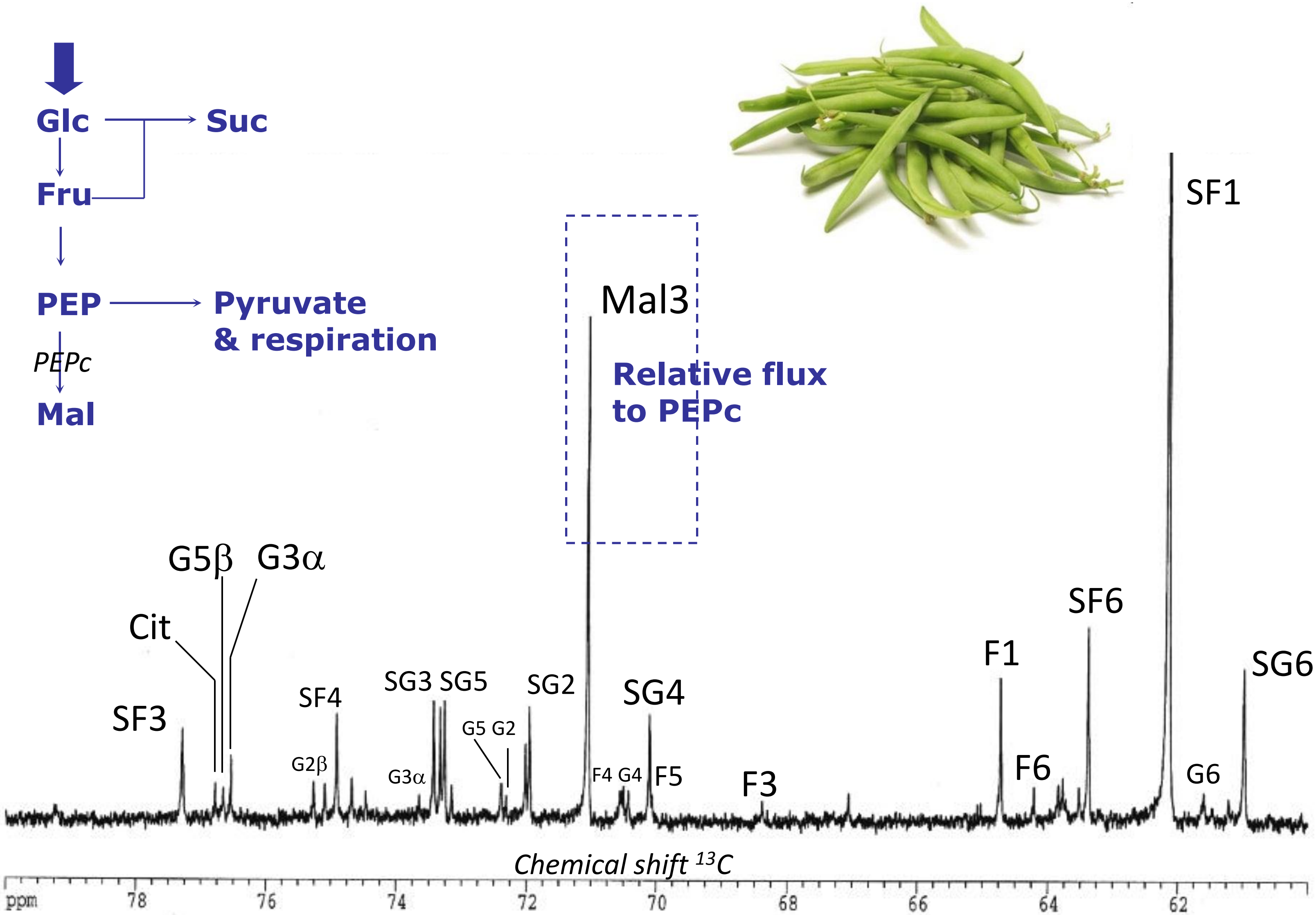
Is complementary to other measurements (e.g. labeling in $CO_2$)

In vivo measurements are possible (with restrictions)

Is not adapted to follow labeling in heavy molecules (e.g. cellulose)
or minor compounds (e.g. cofactors)

# *Example*

*$^{13}$C-1-glucose labeling in bean leaves*

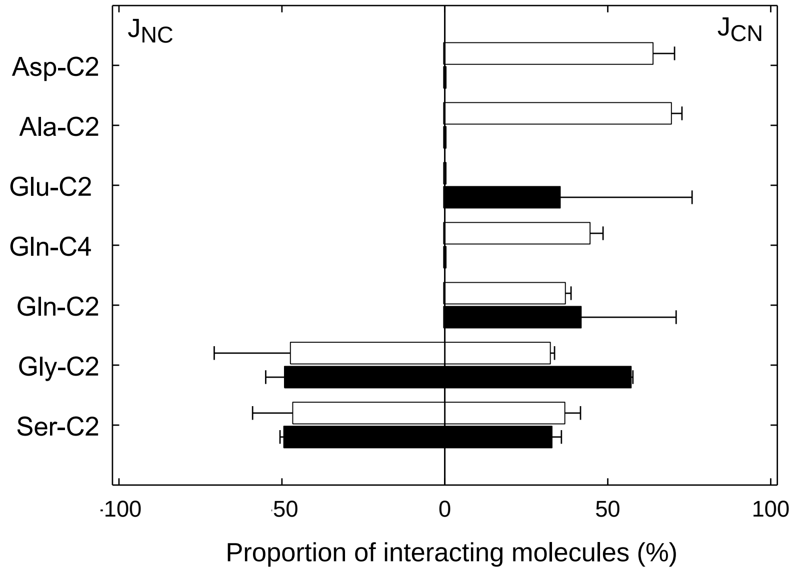


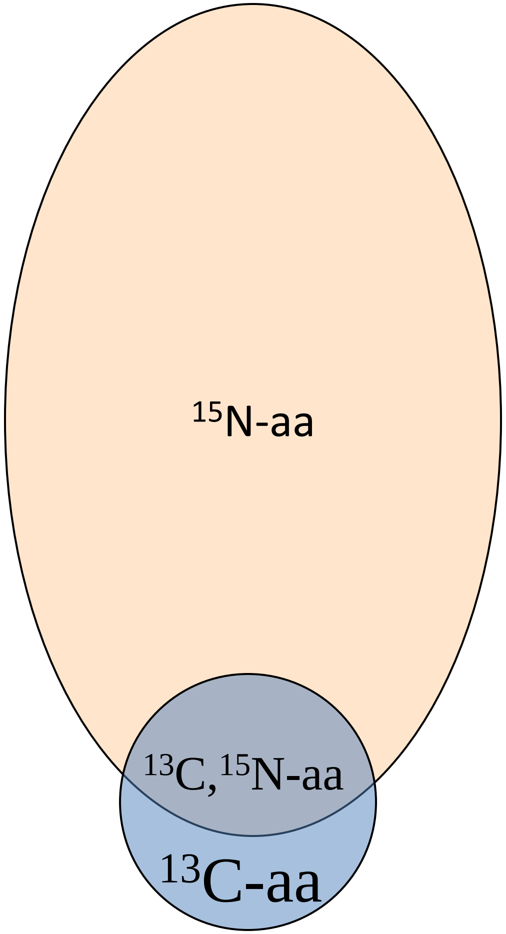
# *Key results obtained in the lab (1)*

## *Double labeling $^{13}C$-$^{15}N$*

% of $^{15}N$-molecules that are also $^{13}C$-labeled

% of $^{13}C$-molecules that are also $^{15}N$-labeled




Most $^{15}N$-labeled molecules are NOT $^{13}C$-labeled, while ca. 60% $^{13}C$-molecules are also $^{15}N$-labeled.

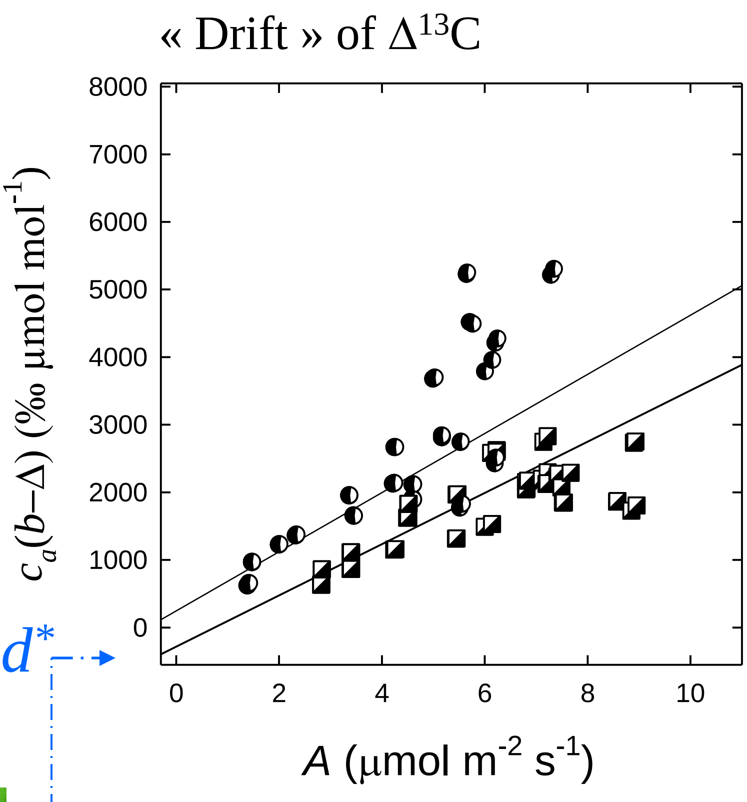
*Most α-cetoglutarate molecules come from $^{12}C$ (« old » carbon): remobilization !*

Gauthier et al. 2010

## *Key results obtained in the lab (2)*

***Natural isotope composition of respiratory $CO_2$ in the light***

*Leaves photosynthesised in an artificial $^{13}C$-depleted $CO_2$ atmosphere*



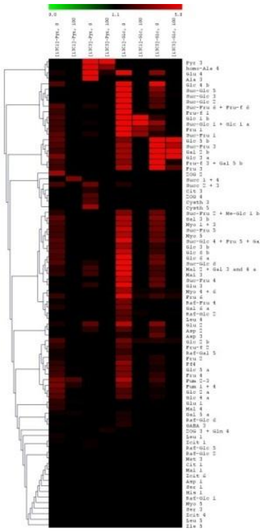

*We see that **e < 0 (respiratory apparent fractionation)**, an « old » carbon soucre is used to produce day-evolved $CO_2$*

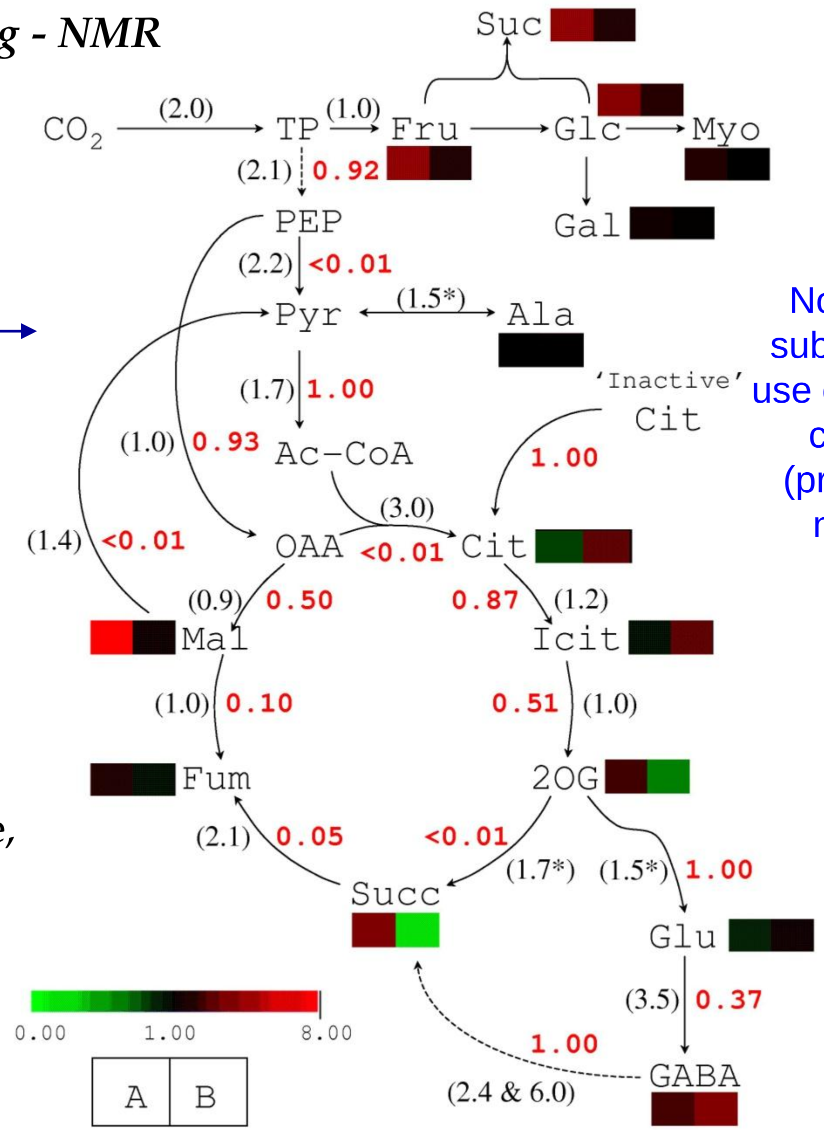
Under certain conditions, $\frac{d^*-f\Gamma^*}{\Gamma-\Gamma^*} = e$

Tcherkez *et al.* 2010, *in press*

## *Key results obtained in the lab (3)*

*Flux measurements after $^{13}CO_2$-labeling - NMR*






Note the substantial use of stored citrate (previous night)

- Slow steps in the light: citrate synthase, 2OGDH

- The 'Krebs cycle' is NOT cyclic

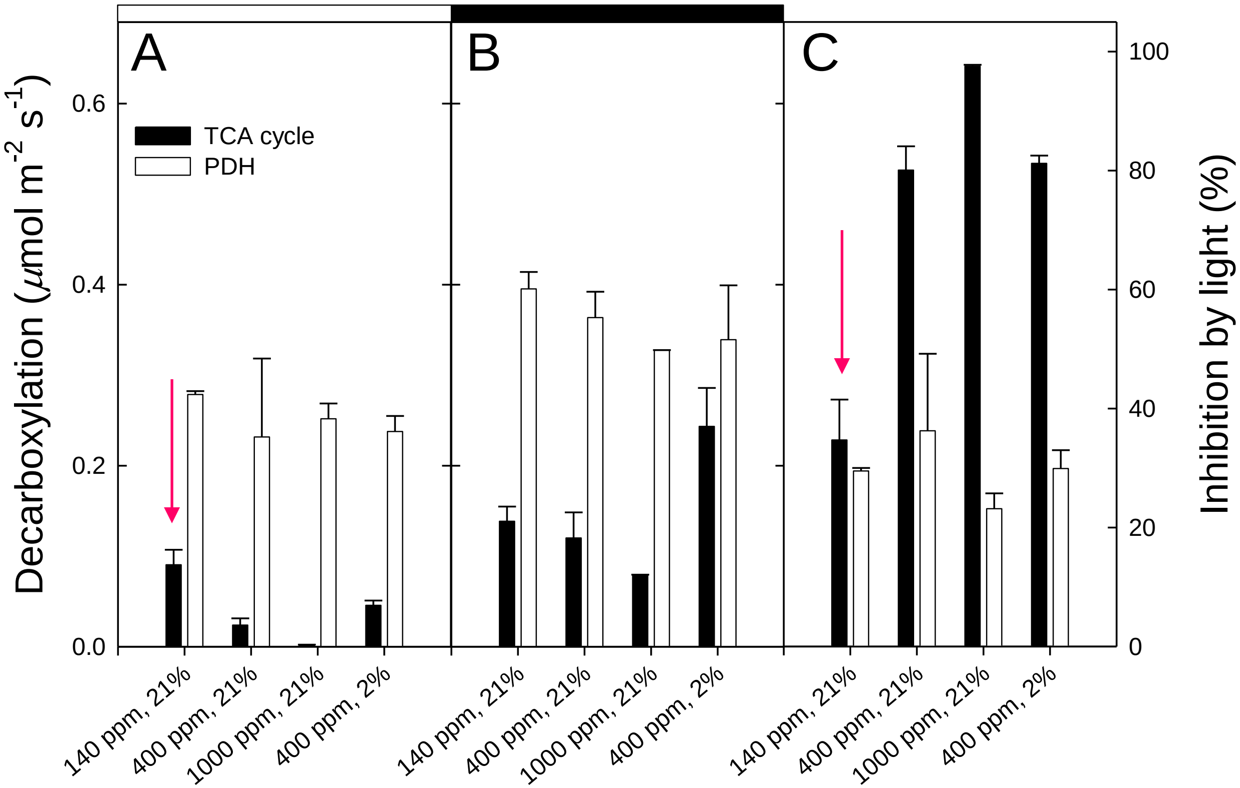
- Remobilisation of « old » citrate

## *Key results obtained in the lab (4)*

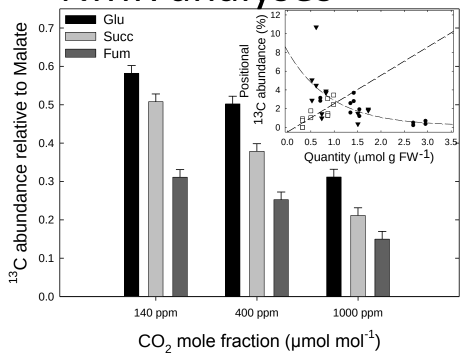
### *In vivo decarboxylation activities*

[13]C-pyruvate labeling and decarboxylating intensity (with $\Delta^{13}C$):

IRMS measurements




NMR analyses

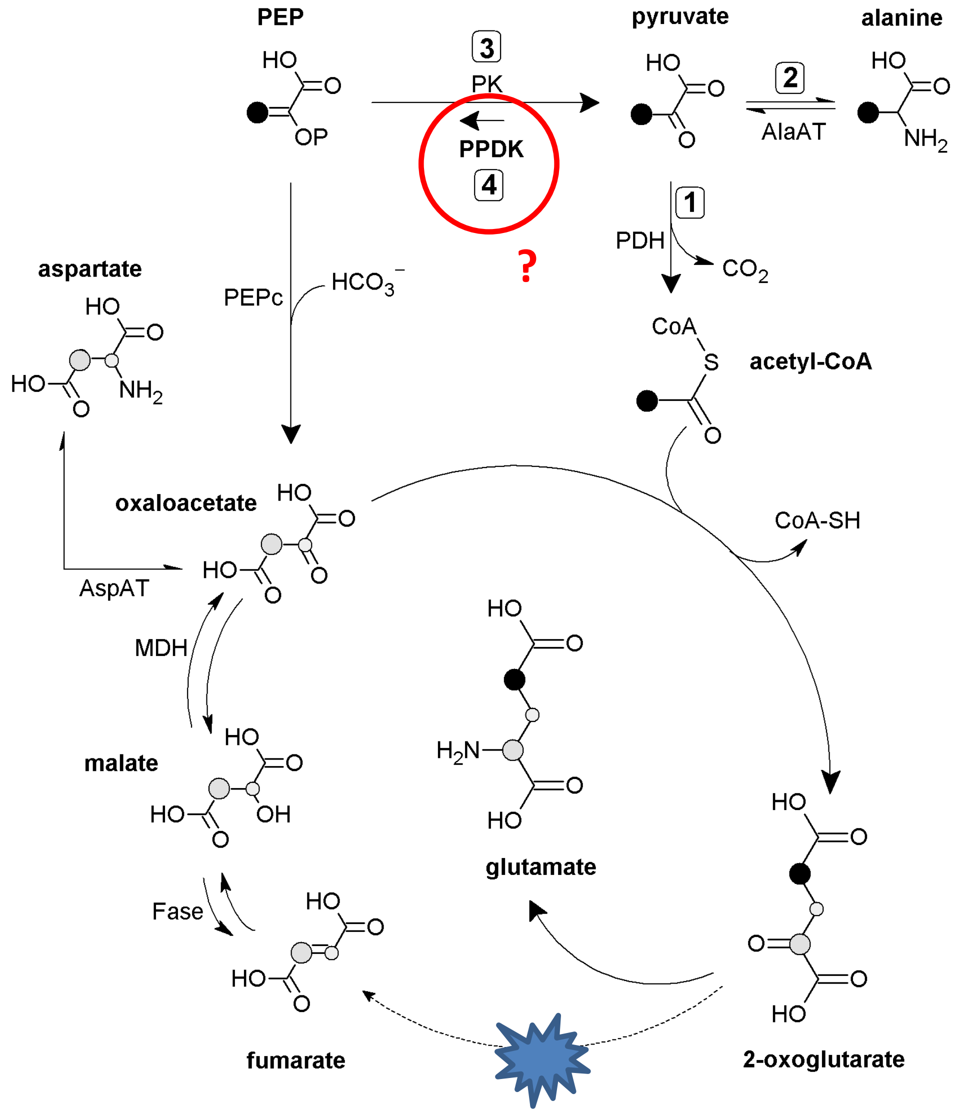



The labeling in key intermediates increases as photorespiration increases

The TCAP is more inhibited under high $CO_2/O_2$ conditions (low photorespiration).

*Photorespiration (at least transiently) increases day respiration.*

# ***Key results (5):*** Pyruvate metabolism



# $^{13}C$-labeling of illuminated leaves

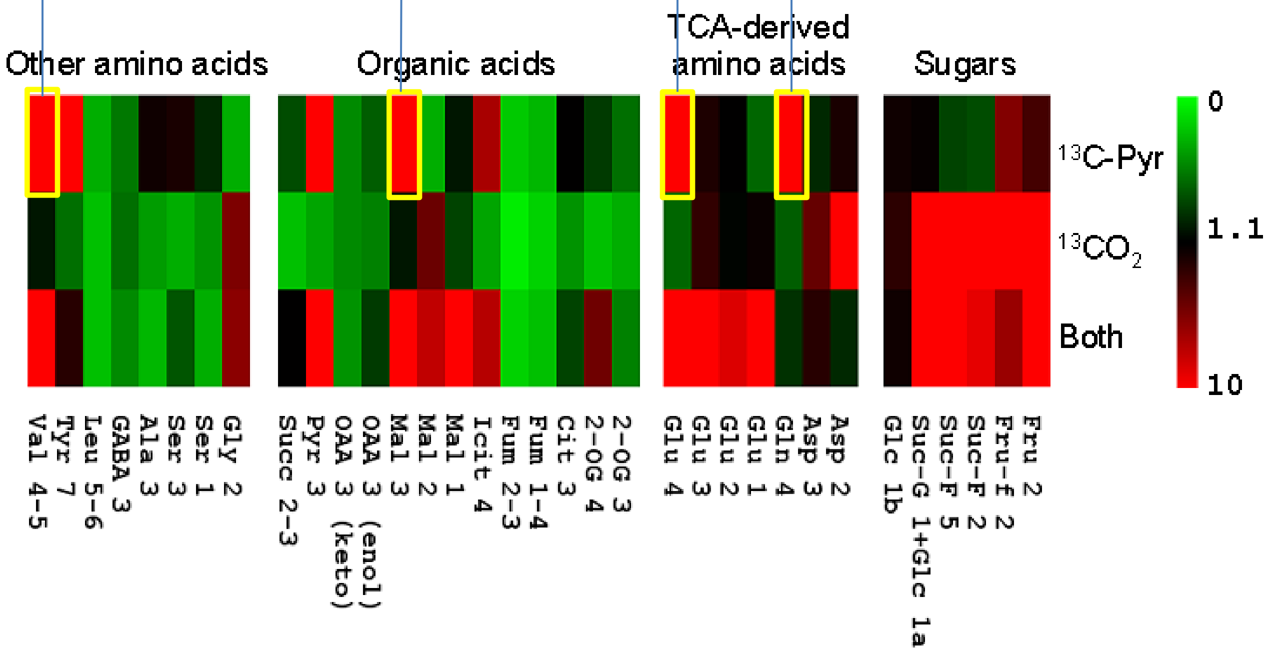
*Cocklebur leaves (X. strumarium)*

Three types of $^{13}C$-labeling
- $^{13}C$-3-pyruvate
- $^{13}CO_2$
- Both

Downstream Pyruvate utilization

Mal-3
Inherited **from PEP** via PEPc-activity

Glu-4
Inherited from 2OG via the Krebs cycle



# $^{13}$C/D-labeling of illuminated leaves

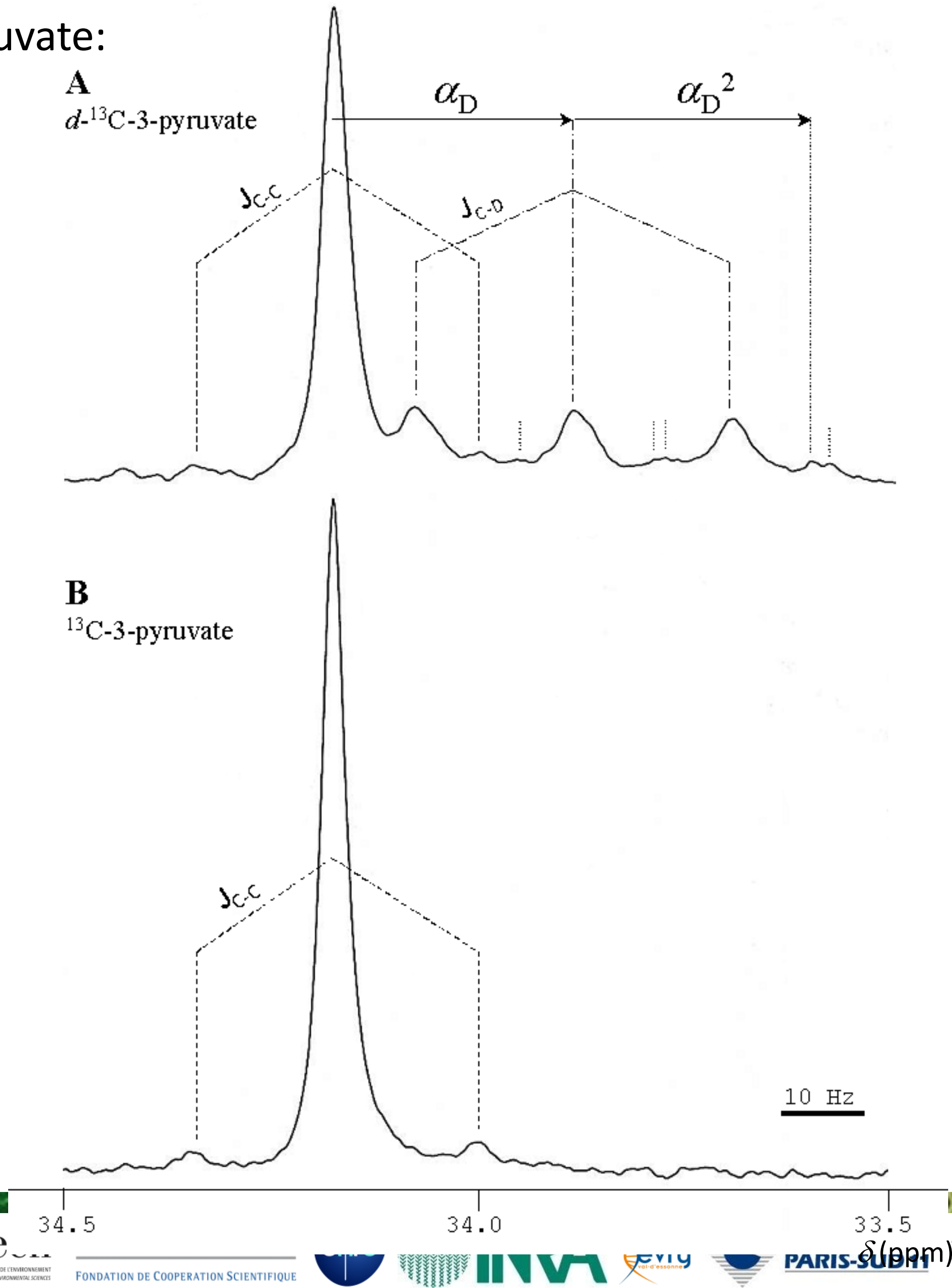
Labeling with $^{2}H$(50%)-$^{13}C$(99%)-pyruvate:

$^{13}$C-pyruvate: $^{13}CH_3$-CO-COOH
*d*-$^{13}$C-pyruvate: $^{13}CD_3$-CO-COOH

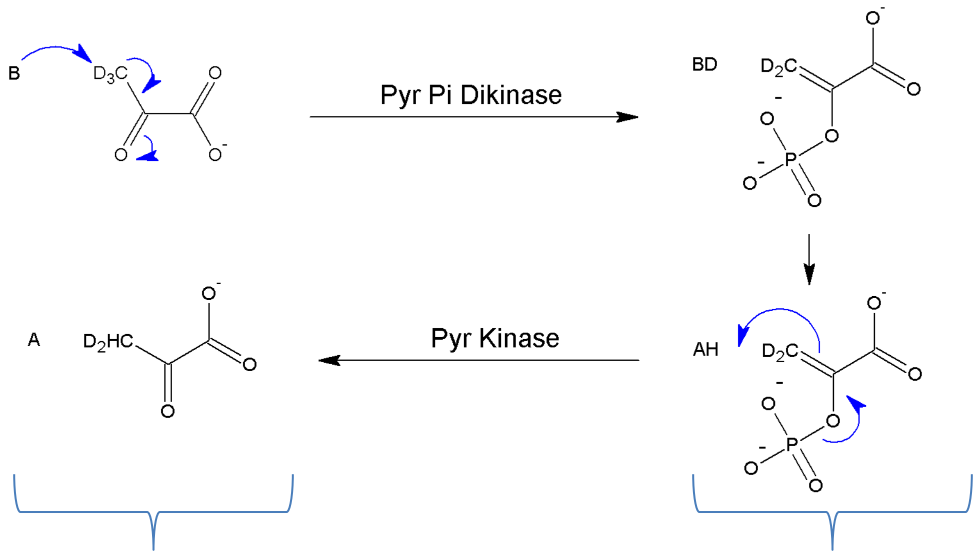
Detection of deuterated $^{13}$C-glutamate C-4

↓

%D of 33%



Proton exchange with the solvent:




Pyruvate

PEP

Going back and forth via PPDK and PK caused the deuterium loss

# *What next?*

## Scientific issues

*How* is respiration and nitrogen assimilation influenced by photorespiration ?

Are other metabolisms implicated in interactions (e.g., $C_1$-metabolism, Asp metabolism, Sulfur metabolism)?

Are there other pathways involved for providing respiratory intermediates?

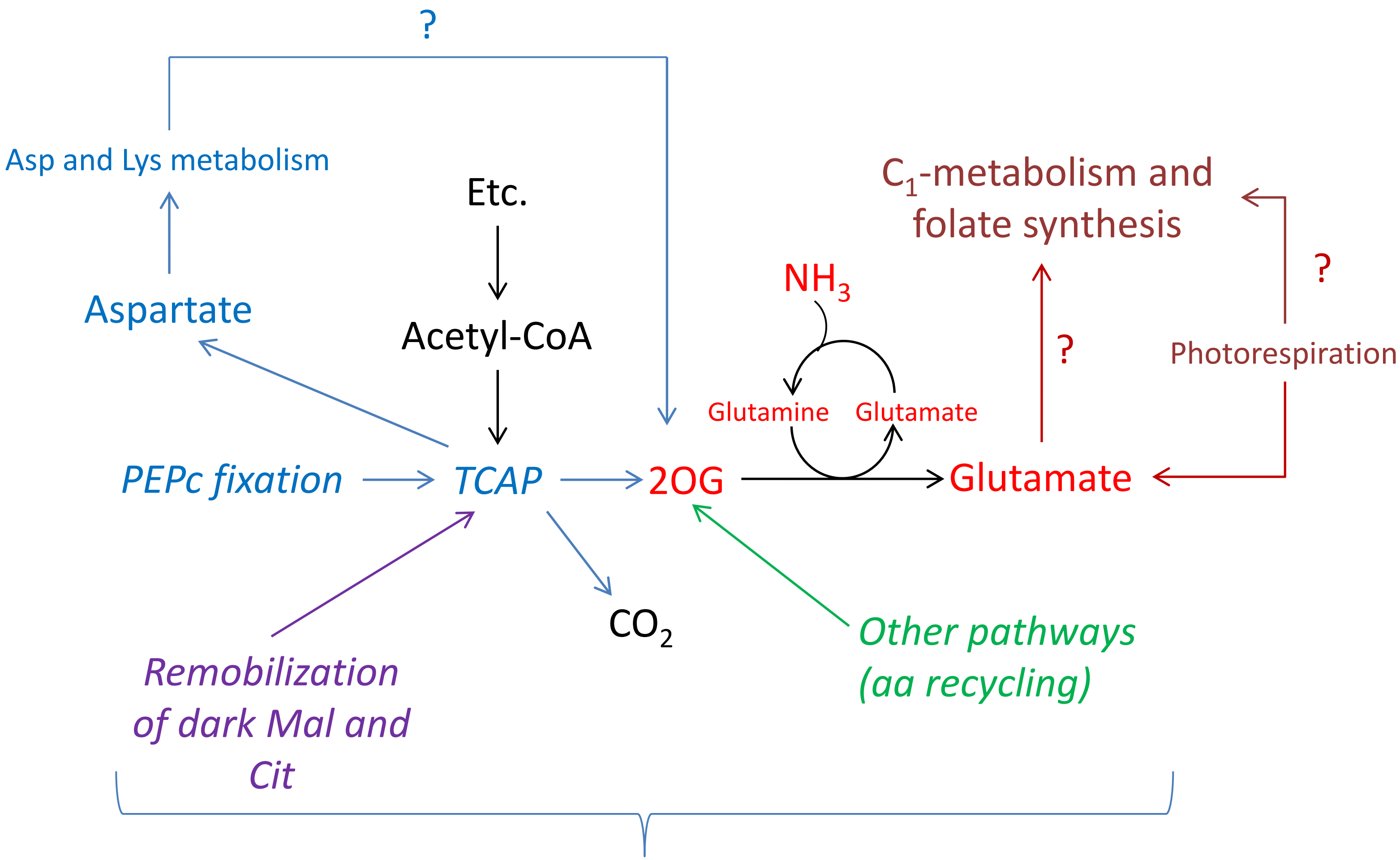
What about post-translational control (phosphorylation, acetylation)?

## Technical issues

**NMR spectrometer**
LabEx: 45% of the budget
55% missing: Sésame

+

Infrastructures (air conditioning, etc.)




*How old are such C sources?*
*Can we quantify contributions?*

## *Methods and means*

*Arabidopsis* mutants in key metabolic steps

Physiological manipulation of photosynthesis and photorespiration ($CO_2/O_2$)

Isotopic methods : labeling, fluxomics, fractionation measurements

Metabolomic characterization

(Phospho)proteomics

## *Human resources committed to the project*

**IBP team (UMR8618)**

Guillaume Tcherkez
Michael Hodges
Mathieu Jossier
Aline Mahé
Bertrand Gakière
Valérie Flesch
**Post-doc LabEx (2012- )**
Edouard Boex-Fontvieille (Post-Doc ANR)
Pierre Petriacq (ATER)
Linda de Bont (PhD student)

**Plateforme Métabolisme-Métabolome (IFR87)**

Françoise Gilard
Caroline Mauve
Florence Guérard
Valérie Cantonny
Marlène Lamothe

The **NMR spectrometer** will integrate the Platform equipment

## *Other resources (not mentioned before)*

Institut Universitaire de France

## *The project per se also included...*

*Remobilisation and nitrogen/sulfur economy*

*Biosynthesis of plant resources*

*"omics" research for improved plant tolerance to stress*

Some aspects are investigated in other SPS Institutes (IJPB, URGV), Flagship projects, Research Axes

Not supported by LabEx-related human resources (post-doc) or equipement

**but**

**Will benefit from fluxomic technologies & expertise at the IBP (platform = facility that offers analytical services to customers)**