Aquatic Primary Production

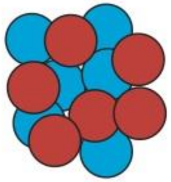
Stable isotopes ($^{13}C/^{18}O$)
Incubations & Methodologies

Ricardo M Letelier

Oregon State University
College of Earth, Ocean, and Atmospheric Sciences

Isotopes

carbon-12

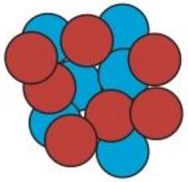


^{12}C

6 protons
6 neutrons

light

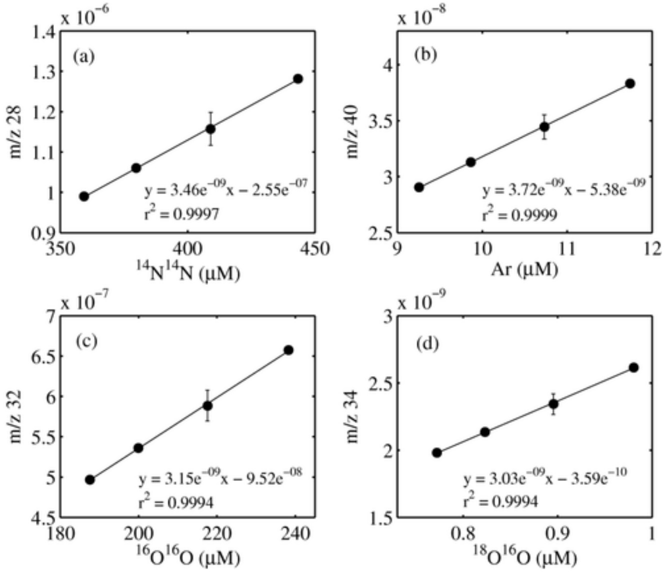
carbon-13



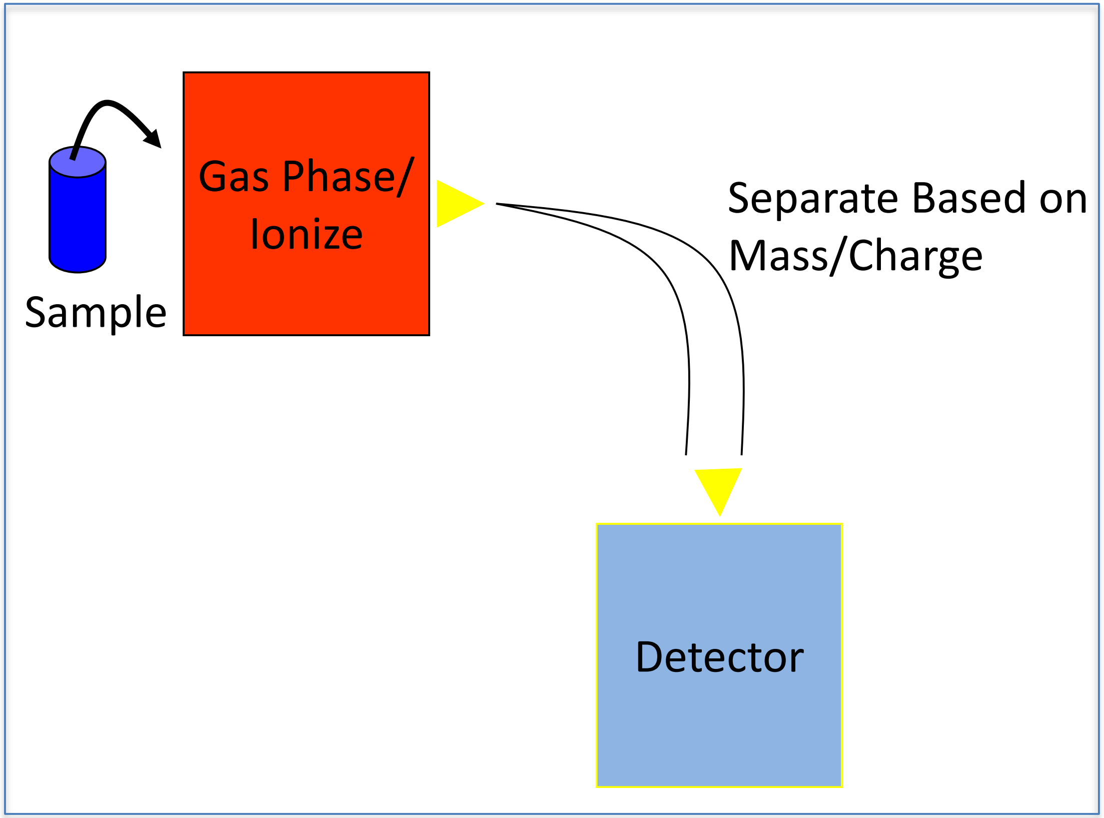
^{13}C

6 protons
7 neutrons

heavy



Mass Spectrometer

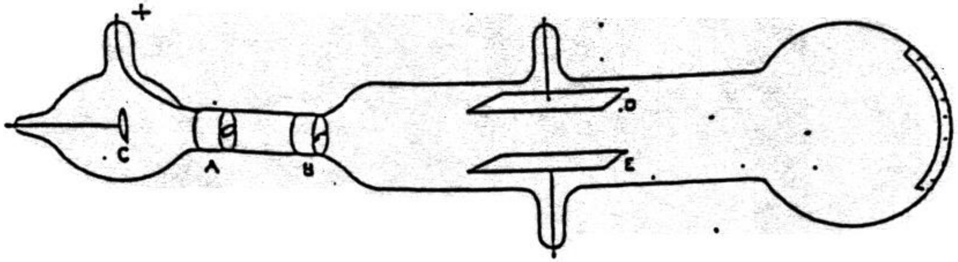


Sample

Gas Phase/ Ionize

Separate Based on Mass/Charge

Detector



Sir Joseph John Thompson (1912)

Early use of ^{18}O and ^{13}C in algal physiology

236 PLANT PHYSIOLOGY

Use of $^{13}C/^{12}C$ and $^{18}O/^{16}O$ in the study of algal photosynthesis and respiration dates back to the 1950s.

- Mehler AH & AH Brown (1952) ^{18}O in photoproduction and consumption.
- Brown AH (1953) ^{18}O and respiration.
- Weis D & AH Brown (1959) ^{18}O and ^{13}C in photosynthesis and respiration in *Ochromonas malhamensis.*

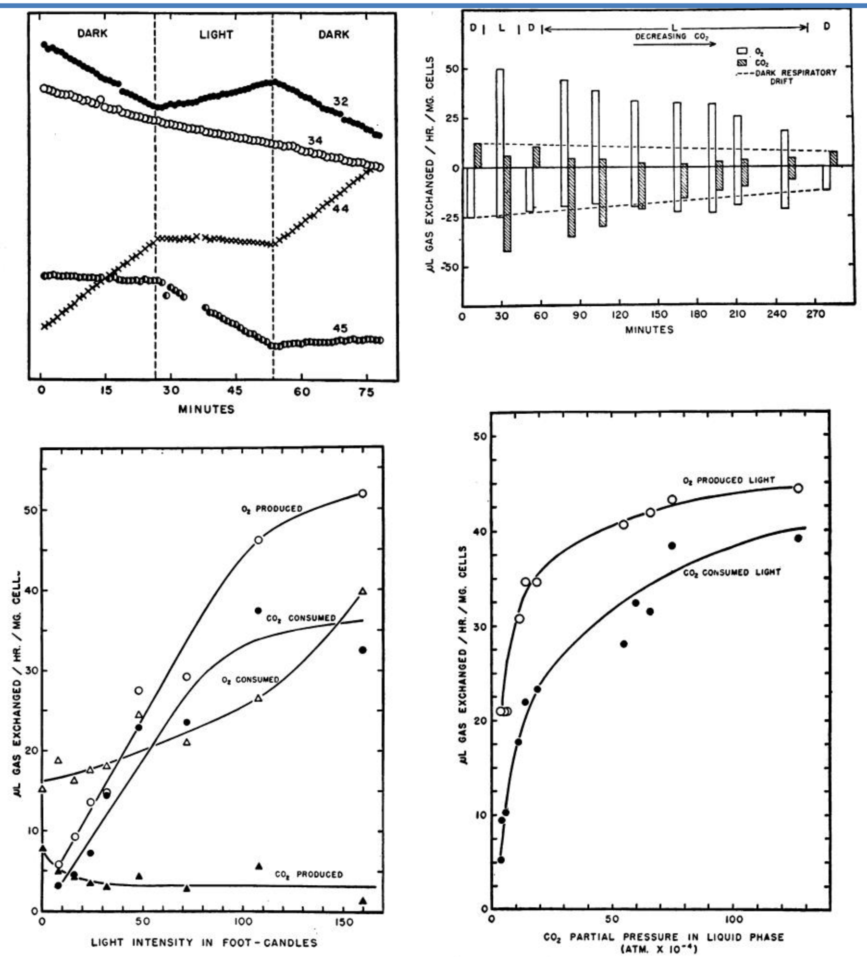


FIG. 1 (*top, left*). Example of mass spectrometric data for 2 isotopic forms of CO_2 (mass 44 and 45) and 2 of O_2 (mass 32 and 34). Ordinate: relative partial pressures of gas.

FIG. 2 (*bottom, left*). The effect of light intensity on gas exchanges by starved cells. Gas phase, $CO_2 : O_2$: He (2 : 3 : 95). Cells starved 24 hours.

FIG. 3 (*top, right*). The effect of decreasing concentration of CO_2 on gas exchanges by starved cells in the light. Red light from 250-watt tungsten lamp filtered through Corning no. 2403 red glass filter. Initial gas phase: $CO_2 : O_2$: He (1 : 3 : 96). Cells starved 18 hours.

FIG. 4 (*bottom, right*). The effect of CO_2 partial pressure on O_2 production and CO_2 consumption by starved cells in the light. Experimental conditions as in figure 3.

Early use of ^{18}O and ^{13}C in algal physiology

Use of $^{13}C/^{12}C$ and $^{18}O/^{16}O$ in the study of algal photosynthesis and respiration dates back to the 1950s.

- Mehler AH & AH Brown (1952) ^{18}O in photoproduction and consumption.
- Brown AH (1953) ^{18}O and respiration.
- Weis D & AH Brown (1959) ^{18}O and ^{13}C in photosynthesis and respiration in *Ochromonas malhamensis.*

236 PLANT PHYSIOLOGY

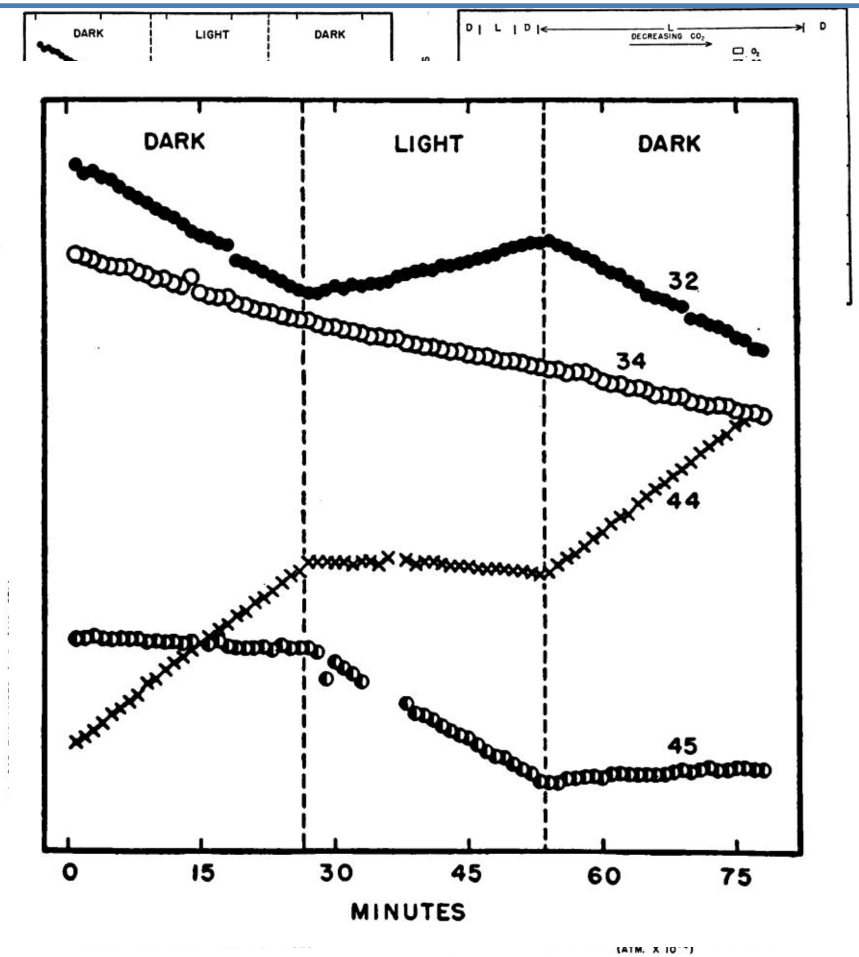


FIG. 1 (*top, left*). Example of mass spectrometric data for 2 isotopic forms of CO_2 (mass 44 and 45) and 2 of O_2 (mass 32 and 34). Ordinate: relative partial pressures of gas.

FIG. 2 (*bottom, left*). The effect of light intensity on gas exchanges by starved cells. Gas phase, $CO_2 : O_2 :$ He (2 : 3 : 95). Cells starved 24 hours.

FIG. 3 (*top, right*). The effect of decreasing concentration of CO_2 on gas exchanges by starved cells in the light. Red light from 250-watt tungsten lamp filtered through Corning no. 2403 red glass filter. Initial gas phase: $CO_2 : O_2 :$ He (1 : 3 : 96). Cells starved 18 hours.

FIG. 4 (*bottom, right*). The effect of CO_2 partial pressure on O_2 production and CO_2 consumption by starved cells in the light. Experimental conditions as in figure 3.

Use of ^{13}C in aquatic primary production

- Incubation method similar to that with ^{14}C (Sample is enriched with $H^{13}CO^{2}$) However, the method requires the determination of 13C/12C in the particulate matter at both t=0 and t=end.

Carbon specific assimilation rate is derived from the change in atom percent ^{13}C

$$\rho = (1/C)\, dC/dt = (a_{t=end} - a_{t=0}) / [(a_i - a_{t=0})(t_{end} - t_0)]$$

ρ = carbon specific accumulation rate derived from isotope measurements
a_i = atom percent ^{13}C of the inorganic carbon (Hama et al. 1983)
$a_{t=0}$ = atom percent ^{13}C of particulate organic matter at t_0
$a_{t=end}$ = atom percent ^{13}C of particulate organic matter at t_{end}

Hence, the photosynthetic rate can be derived from

$$dC/dt = \zeta C \rho$$

Where
ζ = isotope discrimination factor of 1.025 (Hama et al. 1983)
C = the particulate organic carbon concentration

Use of ^{13}C in aquatic primary production

Advantages:

- Can be used in combination with other stable isotopes (^{18}O and ^{15}N)
- Does not require special permits and costly disposal
- Can be used for continuous monitoring of isotopic composition (mass spectrometer may be connected to a gas stream sampled from an experimental chamber and connected via a gas-permeable membrane (Radmer and Hollinger, 1980)
- The mass spectrometer can be coupled with an elemental analyzer (Otsuki et al., 1983; Preston and Owens, 1985) or a gas chromatograph to study the rate of synthesis of specific compounds (Hama et al., 1987, 1988).

Disadvantages:

- Less sensitive than the ^{14}C method
- ^{13}C is present in greater abundance in nature than ^{14}C ($^{13}C/^{12}C$ ~0.0011)
  - requires precise determination of $^{13}C/^{12}C$ at t=0
  - requires a relatively large enrichment of $H^{13}CO_2$ (5-15% of original dissolved inorganic carbon)
- Interpretation of results has the same caveats than those of ^{14}C incubations.

Use of ^{18}O in aquatic primary production

Two approaches:

1) Enrichment of sample with $^{18}O_2$ (^{18}O-^{16}O). Since ^{16}O in H_2O is ~99%, essentially all O_2 evolved during photosynthesis will be ^{16}O-^{16}O (m/z = 32). However, the consumption of $^{18}O_2$ will track the community respiration.

Hence:

$$R = (\delta[^{18}O_2]/\delta t - k[^{18}O_2])\ ([^{18}O_2]+[^{16}O_2])/[^{18}O_2]$$

$$P = (\delta[^{16}O_2]/\delta t - k[^{16}O_2]) + R\ \{[^{16}O_2]/([^{16}O_2] + [^{18}O_2])\}$$

Where:

- $[^{18}O_2]$ and $[^{16}O_2]$ are the mean concentrations of $^{18}O_2$ and $^{16}O_2$ over the incubation period δt.
- $\delta[^{18}O_2]$ and $\delta[^{16}O_2]$ are the concentration changes of $^{18}O_2$ and $^{16}O_2$ over the incubation period δt.
- k is the rate of oxygen consumption by the mass spectrometer (Peltier and Thilbault, 1985)

(from Geider and Osborne, 1992)

Use of ^{18}O in aquatic primary production

Two approaches:

2) Enrichment of sample with $H_2{}^{18}O$. The increase in ^{18}O in O_2 will reflect gross photosynthesis while the net evolution of O2 in the sample will correspond to the net community production (Bender et al., 1987, Grande et al. 1989).

Hence:

$$^{18}\mathrm{O\text{-}GPP} = \left[\frac{^{18}\mathrm{R}(\mathrm{O_2})_{\mathrm{final}} - {}^{18}\mathrm{R}(\mathrm{O_2})_{\mathrm{initial}}}{^{18}\mathrm{R}(\mathrm{H_2O}) - {}^{18}\mathrm{R}(\mathrm{O_2})_{\mathrm{initial}}}\right] \times [\mathrm{O_2}]_{\mathrm{initial}}$$

Where:

$$^{18}\mathrm{R} = \frac{^{18}\mathrm{O}}{^{16}\mathrm{O}} = \frac{m/z\ 34}{(2 \times m/z\ 32) + m/z\ 34}$$

(from Ferron et al., 2016)

Use of ^{18}O in aquatic primary production

In addition, the net change in oxygen can be derived from:

$$\mathrm{NOC} = \left[\frac{(\mathrm{O_2/Ar})_{\mathrm{final}}}{(\mathrm{O_2/Ar})_{\mathrm{initial}}} - 1\right] \times [\mathrm{O_2}]_{\mathrm{initial}}$$

From which a respiration rate can be derived (assuming constancy)

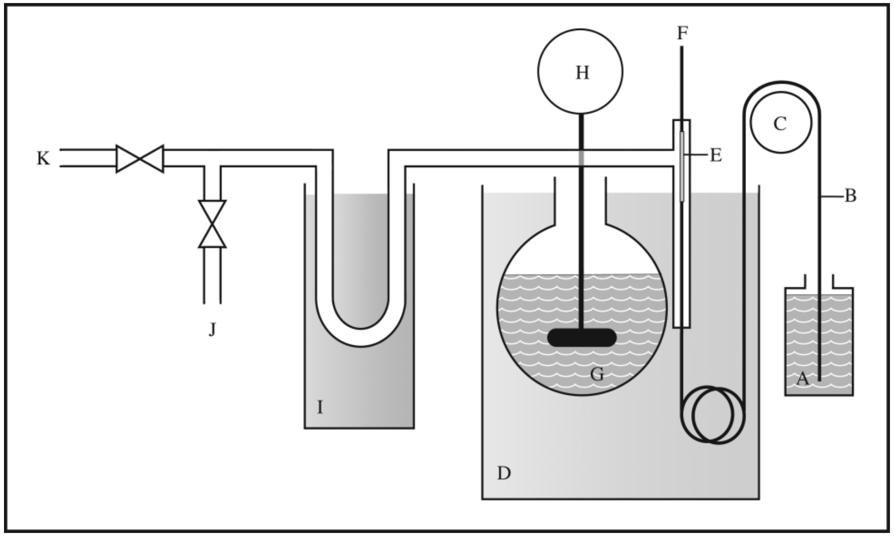
$$\mathrm{CR} = \frac{[^{18}\mathrm{O\text{-}GPP} - \mathrm{NOC}]}{\Delta t}$$

And a Net Community Production

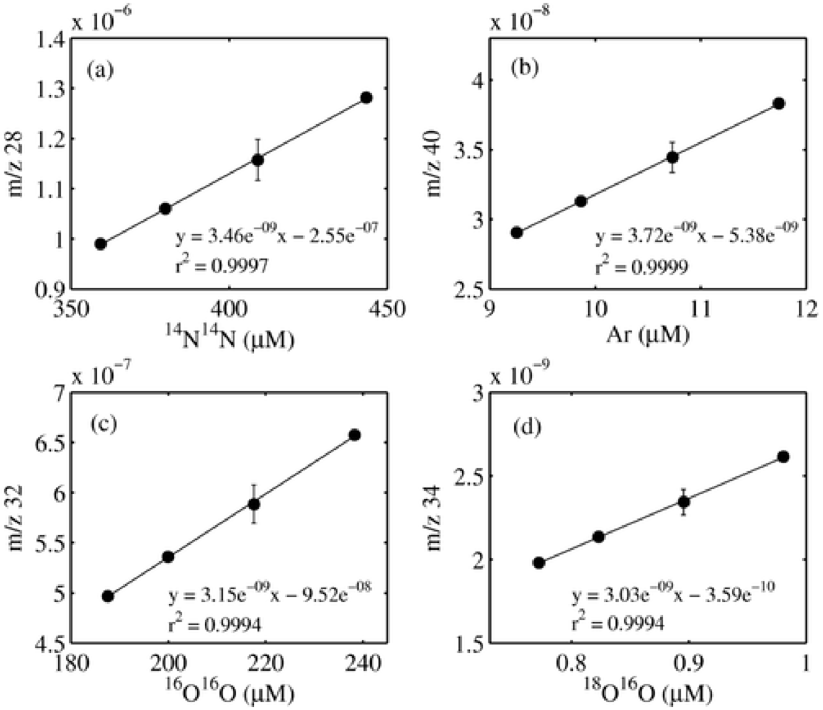
$$\mathrm{NCP} = {}^{18}\mathrm{O\text{-}GPP} - \mathrm{CR}$$

(from Ferron et al., 2016)

Membrane Inlet Mass Spectrometer

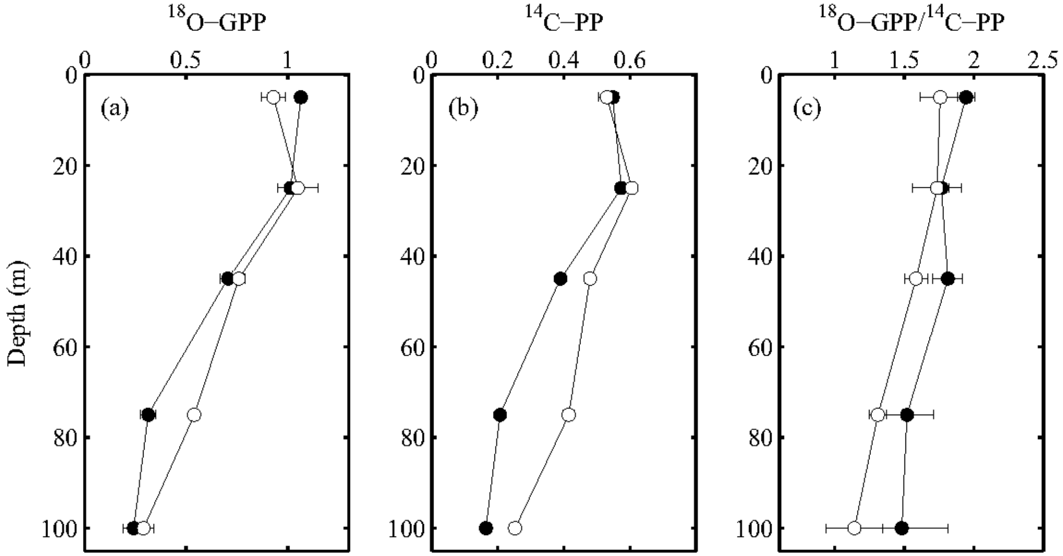


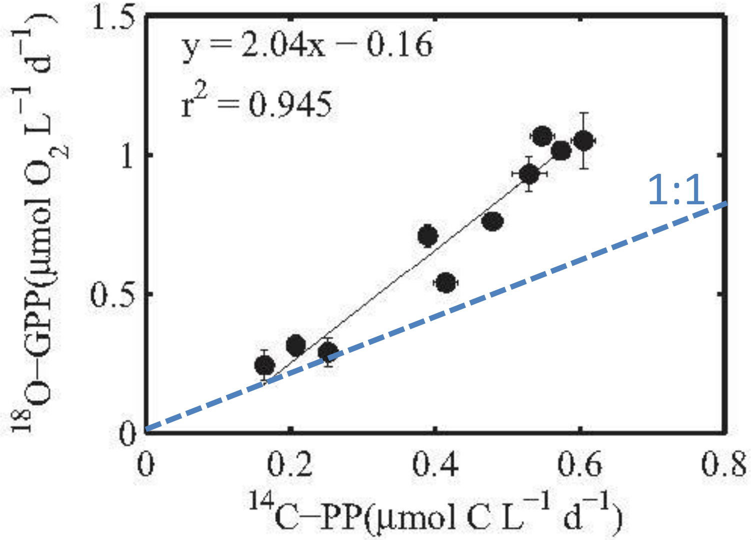
A= sample, B= capillary tube, C= peristaltic pump, D= water bath, E= silicone membrane, F= sample waste, G= flask containing the standard, H= stirrer, I=liquid nitrogen trap, J= connection to vacuum pump, K = connection to quadruple mass spectrometer



Relationship between the QMS signal and the mean expected standard concentration for different isotopes.

Comparison between ^{18}O-GPP and ^{14}C-PP at Station ALOHA

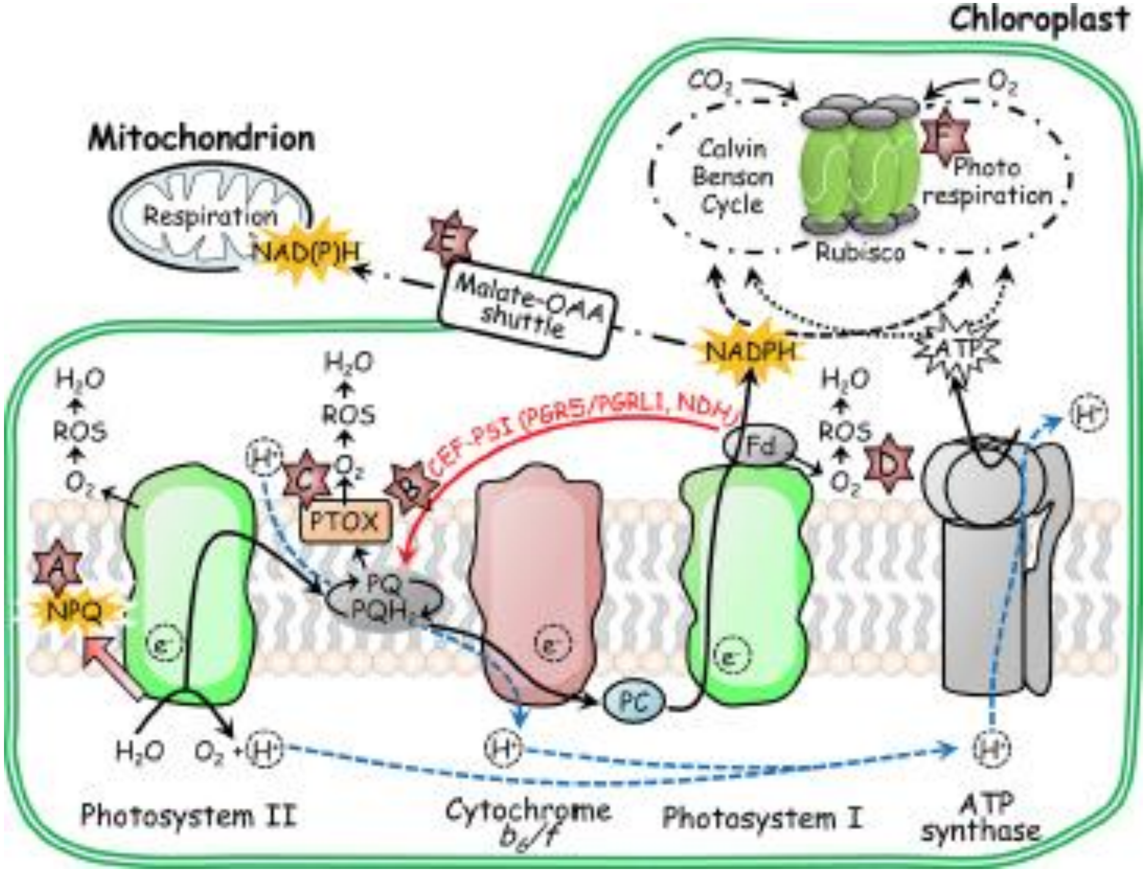




(from Ferron et al., 2016)
See also Quay et al., 2010)

$H_2{}^{18}O$ and the water-water cycle (Asada 1999, 2006)

Excess H^+ and e^- can be removed efficiently by recombining into H_2O in order to avoid photoinhibition



From Yamori, 2016