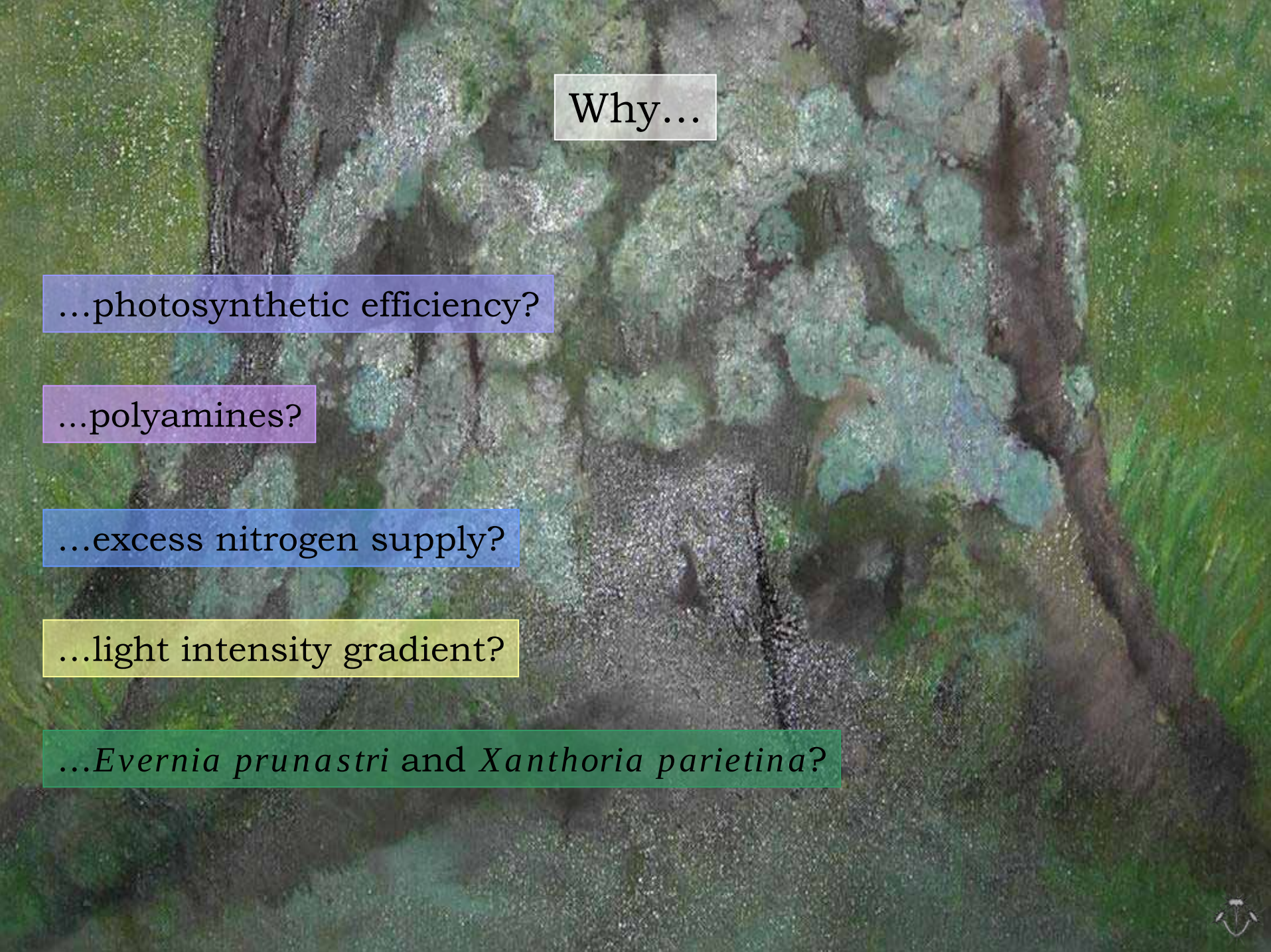
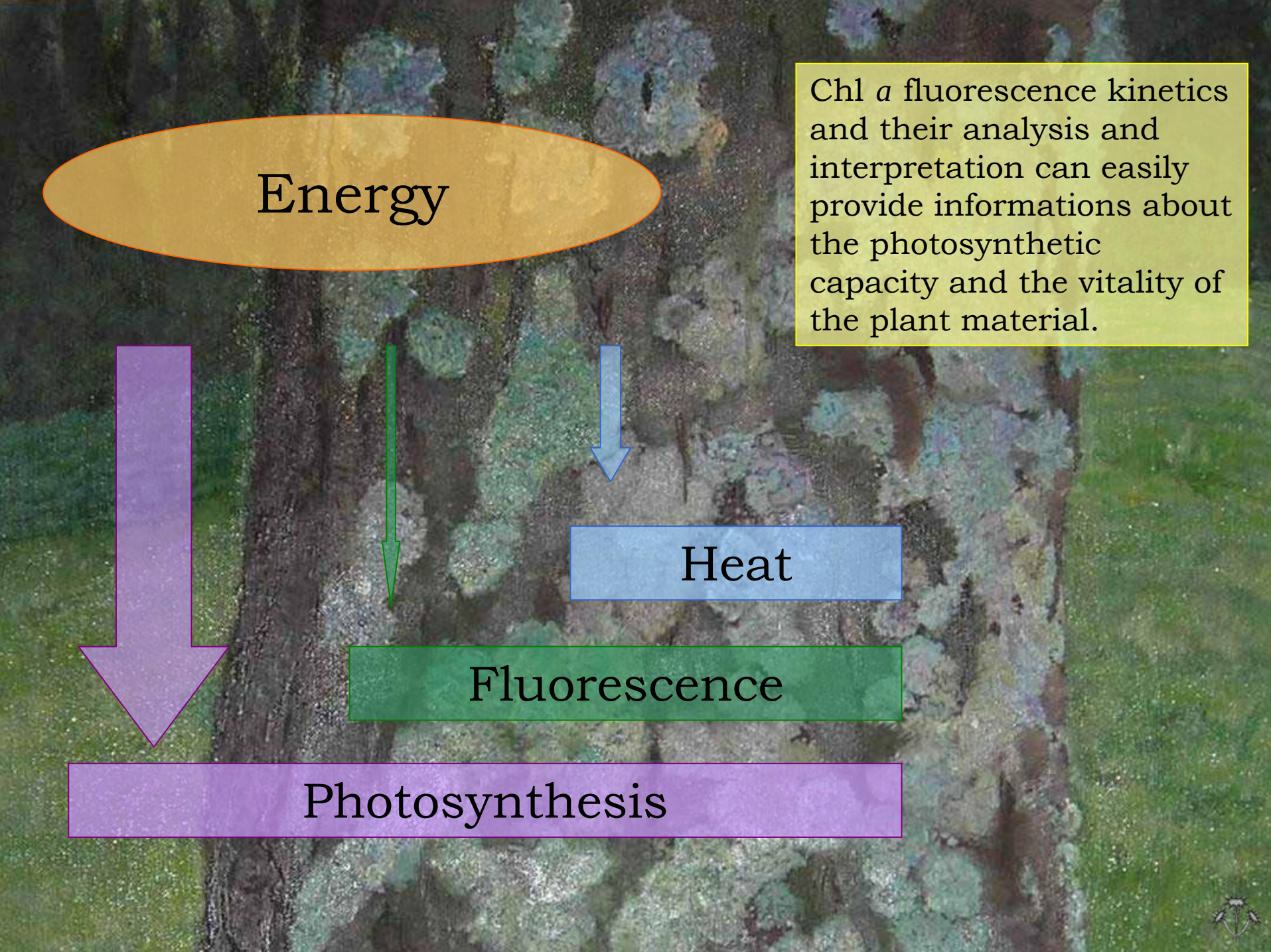
Effects of KNO_3, NH_4NO_3 and $(NH_4)_2SO_4$ on the N-sensitive *Evernia prunastri* and the N-tolerant *Xanthoria parietina* lichens, and the role of polyamines

S. Munzi[1,2], S. Loppi[1], K. Kotzabasis[2], S.A. Pirintsos[2]

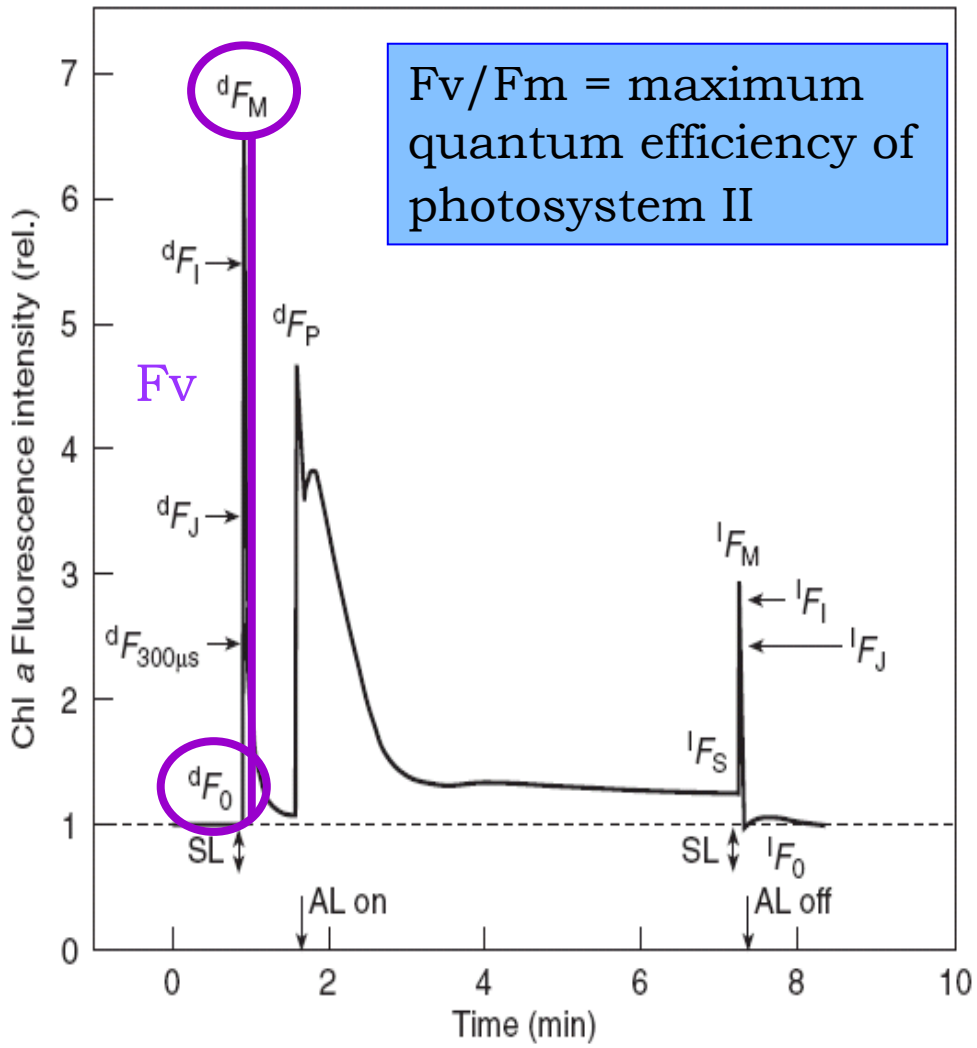
[1]Department of Environmental Science "G. Sarfatti", University of Siena, Italy
[2]Department of Biology, University of Crete, Greece


Why…
…photosynthetic efficiency?
…polyamines?
…excess nitrogen supply?
…light intensity gradient?
…*Evernia prunastri* and *Xanthoria parietina*?



Chl *a* fluorescence kinetics and their analysis and interpretation can easily provide informations about the photosynthetic capacity and the vitality of the plant material.

Photosynthetic efficiency



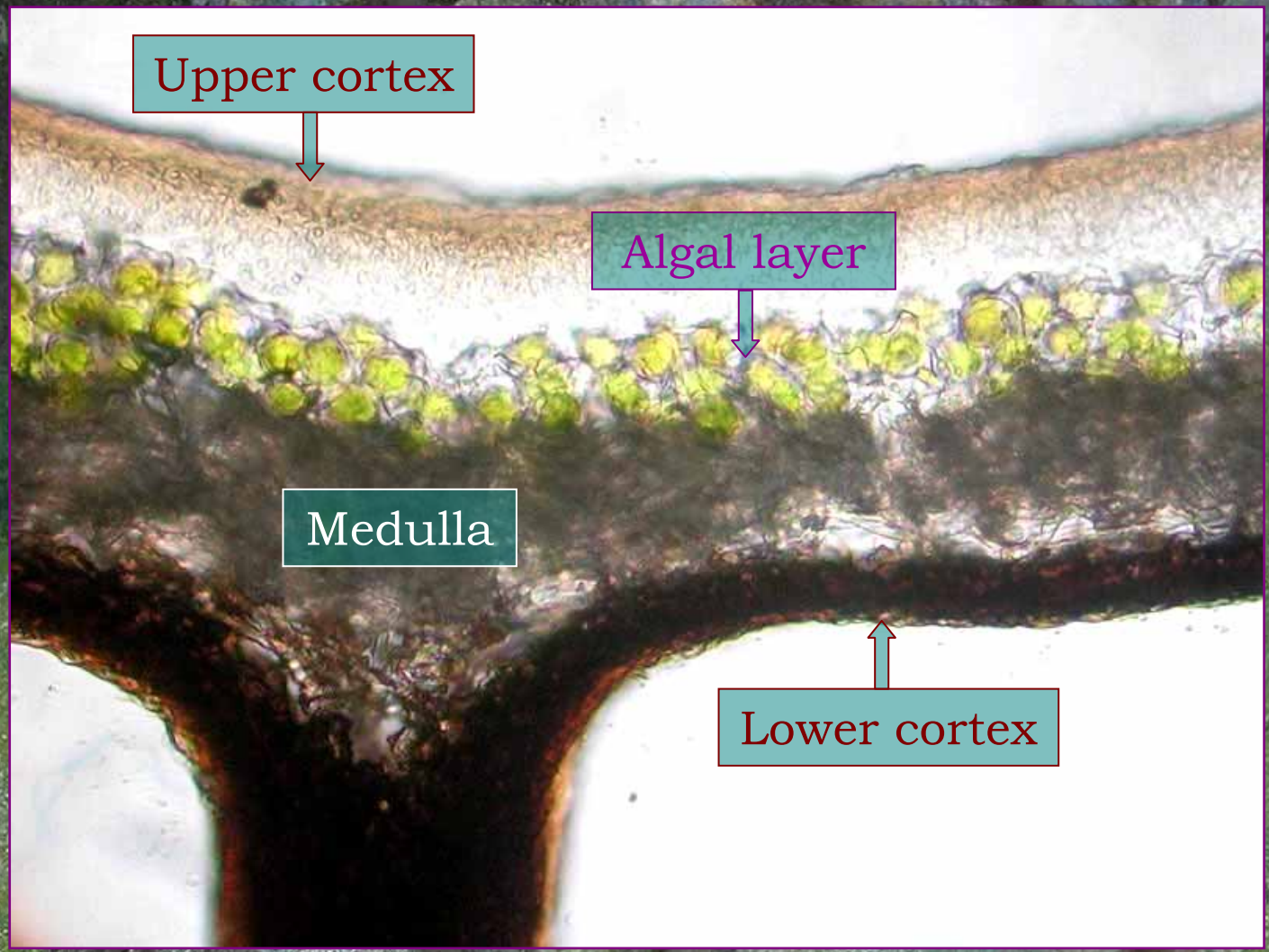
Fv/Fm = maximum quantum efficiency of photosystem II

The fast fluorescence rise starts at the initial low value F_0 and reaches a maximal value F_m.

F_0= initial fluorescence value in a dark-adapted sample

F_m= maximal fluorescence intensity of the sample after a saturating light pulse

$F_v = F_m - F_0$


Upper cortex
Algal layer
Medulla
Lower cortex

Polyamines:

- low molecular weight aliphatic amines
- ubiquitous
- in free form or conjugated to small molecules
- positively charged at physiologic pH

$H_2N\text{-}(CH_2)_4\text{-}NH_2$

Putrescine (PUT)

$H_2N\text{-}(CH_2)_3\text{-}NH\text{-}(CH_2)_4\text{-}NH_2$

Spermidine (SPD)

$H_2N\text{-}(CH_2)_3\text{-}NH\text{-}(CH_2)_4\text{-}NH\text{-}(CH_2)_3\text{-}NH_2$

Spermine (SPM)

Polyamines are involved in a wide range of processes in higher plants:
- growth
- development
- plant-fungi and plant-viruses interactions
- response to microbial symbionts

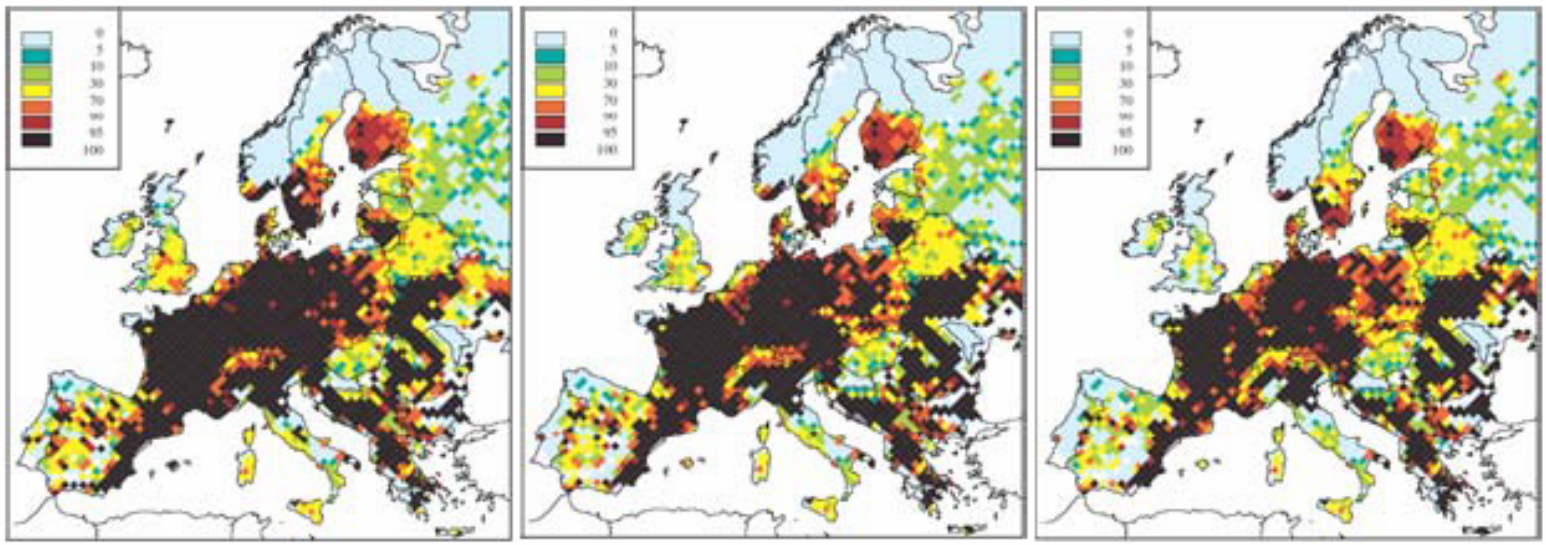
The most important changes in polyamine metabolism are implicated in plant response to environmental challenges and abiotic stressors:

- potassium deficiency
- osmotic-stress
- hypoxia
- heat/chilling
- salt
- ozone
- UV radiation
- heavy metals

An induction of polyamine biosynthesis may confer a stress tolerance

Nitrogen pollution

Excess of critical loads for eutrophication



2000 2010 2020

Light intensity gradient


Evernia prunastri

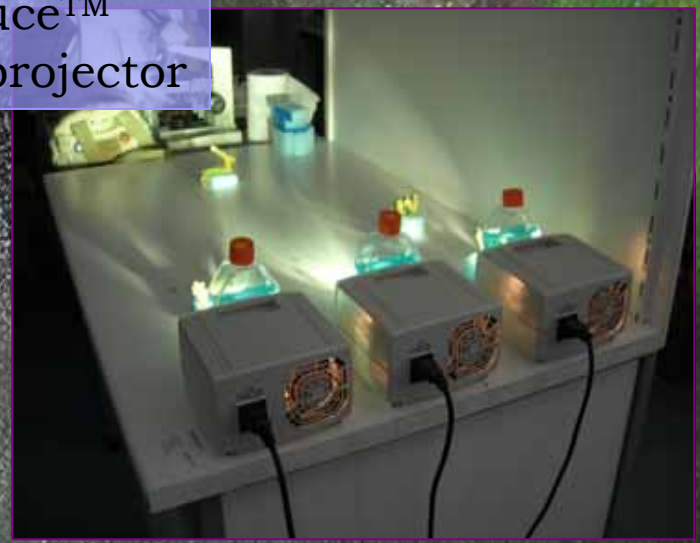

Xanthoria parietina

Treatment with nitrogen compounds

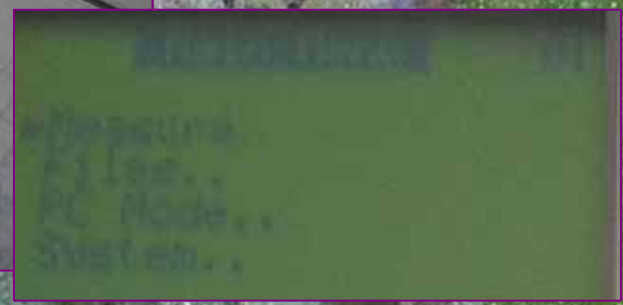
H_2O	
KNO_3	0.05 M
KNO_3	0.1 M
KNO_3	0.5 M
KNO_3	1 M
NH_4NO_3	0.05 M
NH_4NO_3	0.1 M
NH_4NO_3	0.5 M
NH_4NO_3	1 M
$(NH_4)_2SO_4$	0.025 M
$(NH_4)_2SO_4$	0.05 M
$(NH_4)_2SO_4$	0.25 M
$(NH_4)_2SO_4$	0.5 M

Exposure simulating a light intensity gradient

3 h at 40, 150, 700 and 1500 µM/m^2/sec PAR intensities, obtained by a Mille Luce™ M1000 (Stocker & Yale) projector



Handy PEA (Plant Efficiency Analyser, Hansatech)



Mean (N=24) Fv/Fm values (±SD) of the lichen *Evernia prunastri* after 1 and 24 h from treatment with various concentrations of different N-containing solutions.

	after 1 h	after 24 h
H_2O	0.642±0.021	0.656±0.025
KNO_3 0.05 M	0.656±0.019	0.641±0.020
KNO_3 0.1 M	0.641±0.045	0.636±0.018
KNO_3 0.5 M	0.589±0.038	0.547±0.067
KNO_3 1 M	0.628±0.040	0.597±0.031
NH_4NO_3 0.05 M	0.649±0.021	0.647±0.020
NH_4NO_3 0.1 M	0.663±0.019	0.533±0.080
NH_4NO_3 0.5 M	0.522±0.072	0.033±0.025
NH_4NO_3 1 M	0.020±0.017	0.005±0.004
$(NH_4)_2SO_4$ 0.025 M	0.648±0.028	0.647±0.024
$(NH_4)_2SO_4$ 0.05 M	0.677±0.024	0.639±0.035
$(NH_4)_2SO_4$ 0.25 M	0.612±0.039	0.071±0.071
$(NH_4)_2SO_4$ 0.5 M	0.130±0.096	0.029±0.019

Mean (N=24) Fv/Fm values (±SD) of the lichen *Xanthoria parietina* after 1 and 24 h from treatment with various concentrations of different N-containing solutions.

	after 1 h	after 24 h
H_2O	0.547±0.063	0.489±0.086
KNO_3 1 M	0.514±0.064	0.499±0.076
NH_4NO_3 0.05 M	0.526±0.050	0.513±0.074
NH_4NO_3 0.1 M	0.565±0.038	0.538±0.043
NH_4NO_3 0.5 M	0.518±0.106	0.446±0.087
NH_4NO_3 1 M	0.386±0.142	0.495±0.094
$(NH_4)_2SO_4$ 0.025 M	0.522±0.060	0.541±0.054
$(NH_4)_2SO_4$ 0.05 M	0.554±0.064	0.548±0.056
$(NH_4)_2SO_4$ 0.25 M	0.558±0.035	0.504±0.125
$(NH_4)_2SO_4$ 0.5 M	0.416±0.101	0.478±0.110

Mean (N=24) Fv/Fm values (±SD) of the lichen *Evernia prunastri* after 1, 24 and 48 h from treatment with 1mM polyamines and 0.5 M $(NH_4)_2SO_4$.

	after 1 h	after 24 h	after 48 h
Negative control	0.023±0.028	0.022±0.015	0.040±0.005
Putrescine	0.049±0.051	0.610±0.024	0.637±0.032
Spermine	0.030±0.037	0.632±0.031	0.628±0.029
Spermidine	0.619±0.023	0.654±0.022	0.162±0.078

Mean (N=4) Fv/Fm values (±SD) of the lichen *Xanthoria parietina* after 1, 24 and 48 h from treatment with 1mM inhibitors of polyamine production and 0.5 M $(NH_4)_2SO_4$.

	after 1 h	after 24 h	after 48 h
Negative control	0.567±0.046	0.603±0.020	0.555±0.038
Inib. PUT	0.457±0,141	0.481±0.120	0.366±0.105
Inib. SPM	0.415±0.080	0.361±0.192	0.256±0.091
Inib. SPD	0.398±0.151	0.384±0.072	0.285±0.113

SUMMARY

1) The N-tolerant lichen species *X. parietina* is hardly influenced by nitrogen compounds

2) Ammonium can adversely affect chlorophyll *a* fluorescence of the N-sensitive species *E. prunastri*, while nitrate has no effect

3) The effect of NH_4^+ is evident 1 h after treatment only at the highest concentrations tested, while 24 h are necessary to measure effects also at lower concentrations

4) External supply of polyamines dramatically changes the sensitivity of *E. prunastri* to N stress, with spermidine acting immediately, and putrescine and spermine only after 24 hours

5) Treatment with inhibitors of polyamine production reduces the N-tolerance of *X. parietina*

FUTURE RESEARCH

1) Completing the experimental design, testing the effect of polyamines and their inhibitors also with NH_4NO_3
2) Checking the effect of treatments up to 96 h from incubation
3) Measuring the concentration of endogenous polyamines before and after treatment

The End

Many thanks.

rebecca filippi
"le muffe"
104x84 olio su tela,2006