



Towards an index of biological state of the soil as a new tool for ecotoxicological studies

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Towards an index of biological state of the soil as a new tool for ecotoxicological studies

Christian Mougin, Karine Laval

Salima Taïbi, Daniel Tessier, Anne-Sophie Lemaire, Sylvie Baray



The context: ecotoxicology of the soils

Assess the fate and impact of pollutants on the soil ecosystem

- the cropped soils
(plant protection products, medicines, EDCs, ETM...)

Take into account the other perturbations of these ecosystems

- anthropic: practices, management, wastes
- natural: pedo-climatic

Question:

**What should be an equilibrated state of the soil ecosystem
(a situation of « reference ») useful to assess the real
impact of pollutants ?**

Our research program

« An index of biological state of the soils »



3 objectives:

- build an index for natural (meadow) and cropped soils
- study its variation in response to agronomic (practices at the field) or environmental (pollutions in microcosms) constraints
- validate it *in situ* using fields, polluted soils... exhibiting various physico-chemical and biological properties

Our hypotheses

- The biological state of a soil can be assessed by a mathematical combination of variables, describing both the structural and functional diversity of the microbial communities
- The multiplicity of the determined variables allows the modulation of the bias related to each method
- Meadows and crops behave as systems of reference regarding polluted sites

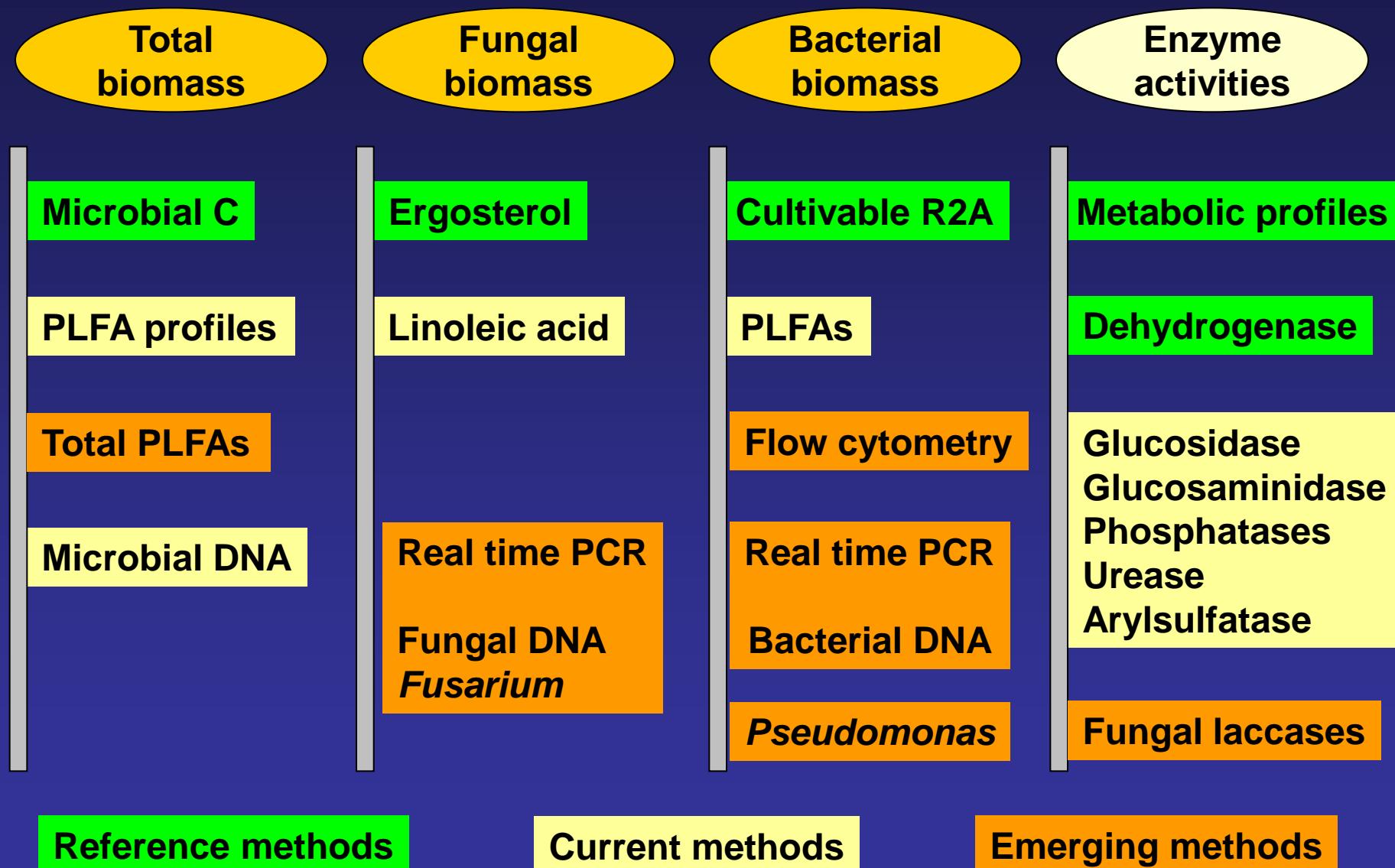
Our strategy

The description of the relationships between physico-chemical and biological variables in contrasted situations

Three steps linked by mathematical modelling:

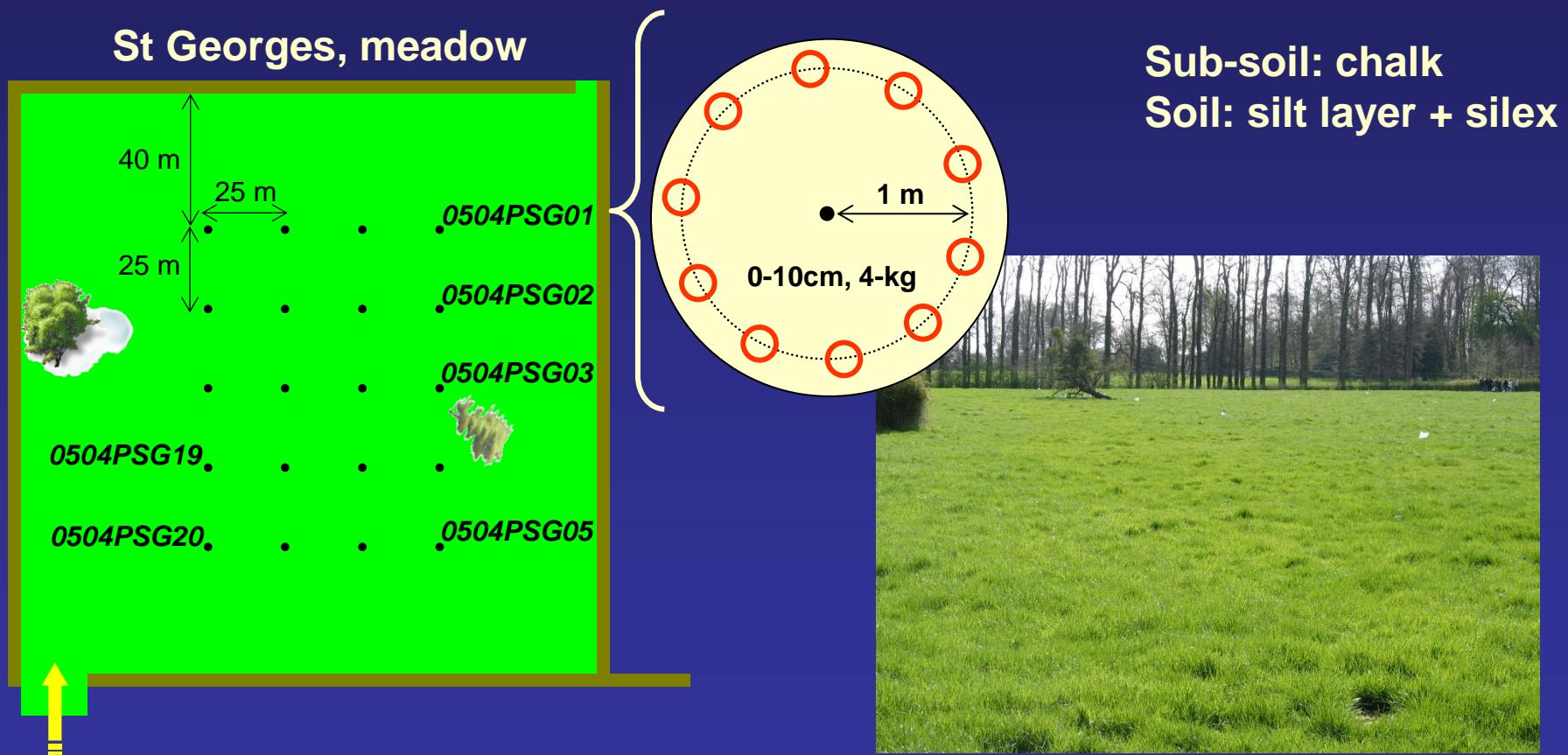
- **measure of the variables in agronomic situations (year 1)**
meadows and crops
- **assessment of the variations due to pollution (year 2)**
microcosms, organics, ETM
- **validation *in situ* in fields, polluted soils... (year 3)**

Variables describing the microbial communities



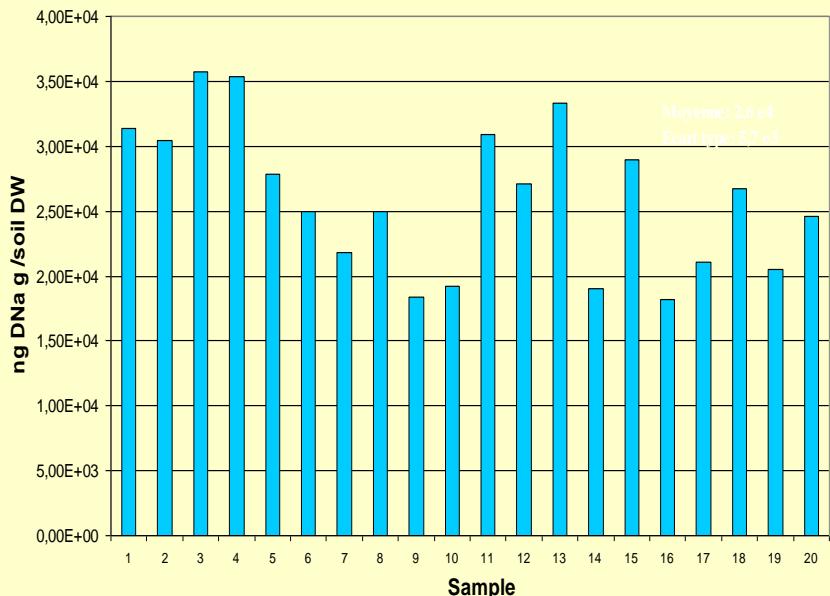
Measure of the variables at the field

- 2 sites: St Georges and Yvetot (near Rouen)
 - 2 situations: meadows and crops
 - 3 periods of sampling: april, june and september



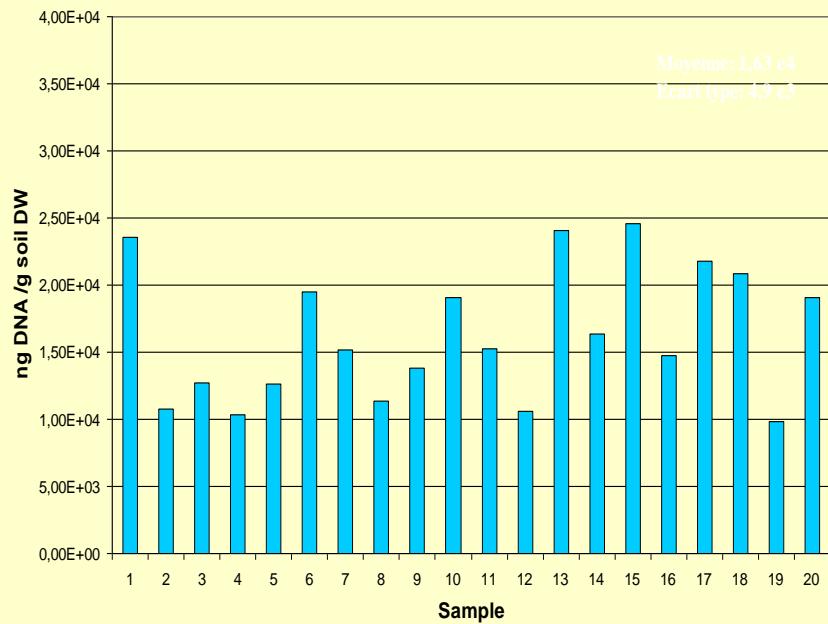
St Georges: total biomass, microbial DNA

Meadow



$26.0 \pm 5.7 \mu\text{g/g}$

Crop: wheat



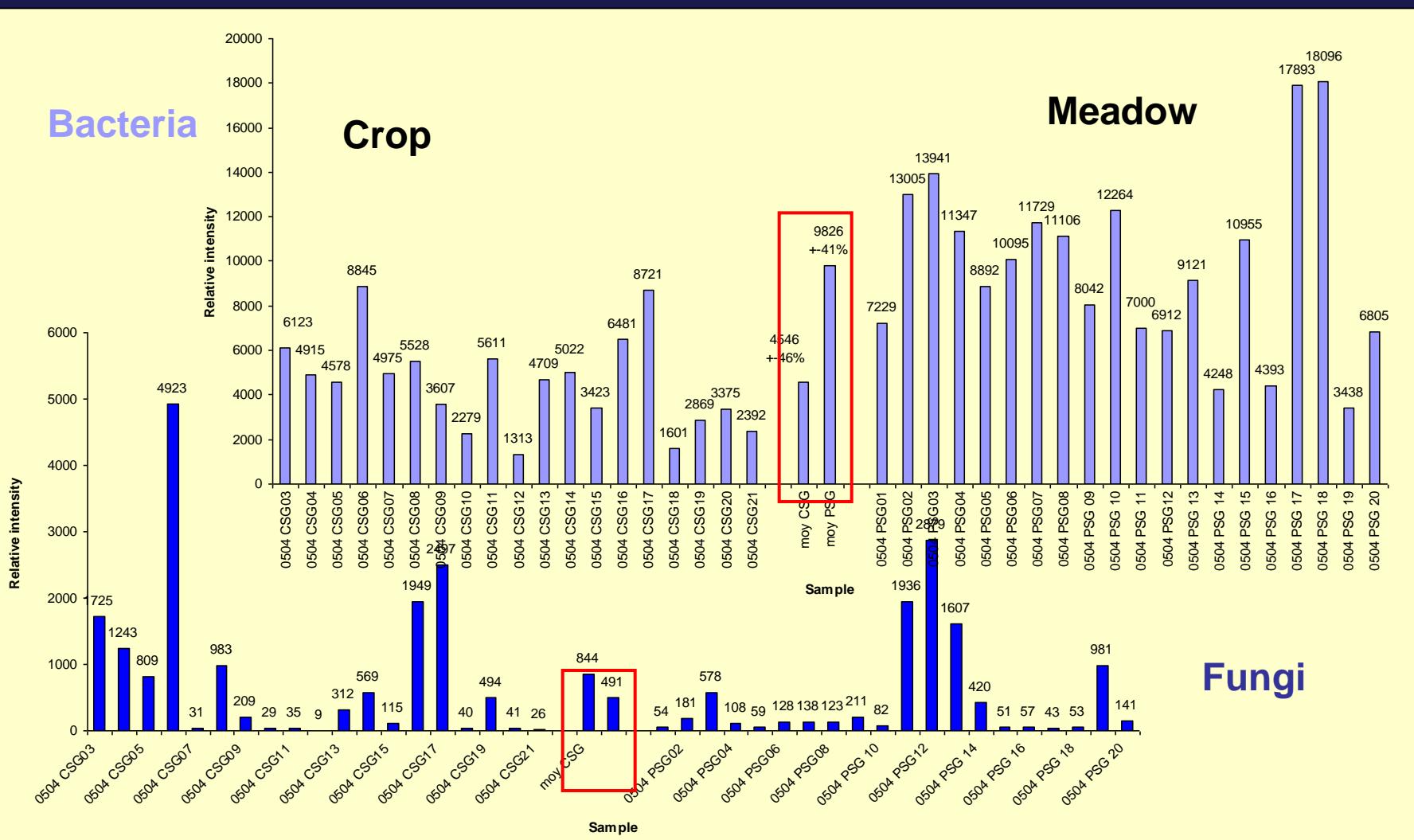
$16.3 \pm 4.9 \mu\text{g/g}$

- ▶ higher total microbial biomass in the meadow, with a lower spatial variation

Structural diversity: partial results

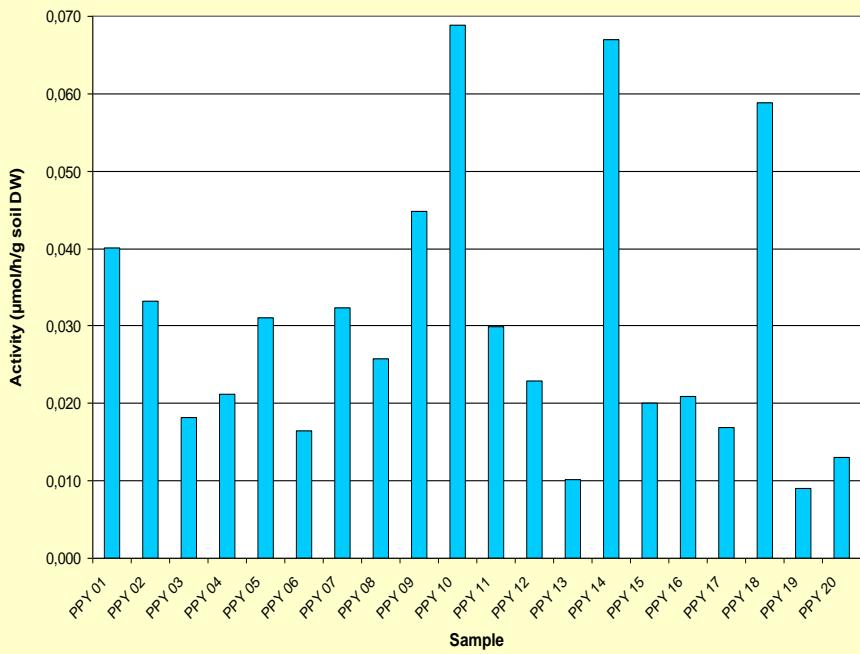
Variable	St Georges meadow	St Georges crop	Yvetot meadow	Yvetot crop
Microbial DNA (µg/g soil DW)	26.0 ± 5.7	16.3 ± 4.9	36.0 ± 4.9	15.6 ± 2.1
Fungal DNA (ng/g soil DW)	201.0 ± 139.0	18.7 ± 8.5	118.0 ± 41.8	33.4 ± 8.4
Ergosterol (µg/g soil DW)	12.2 ± 3.3	3.4 ± 0.6	4.7 ± 1.9	1.6 ± 0.5
<i>Fusarium</i> DNA (ng/g soil DW)	0.7 ± 0.5	0.2 ± 0.2	1.7 ± 0.6	0.5 ± 0.2
Cultivable bac. (bact/g soil DW)	4.10^8	5.10^8	$1,5.10^9$	5.10^8
Flow cytometry (bact/g soil DW)	$5.0.10^9 \pm 1.1.10^9$	$2.8.10^9 \pm 7.6.10^8$	$1.2.10^{10} \pm 7.3.10^9$	$3.0.10^9 \pm 1.8.10^9$
<i>Pseudomonas</i> (bact/g soil DW)	7.10^6	$1.3.10^7$	$2.6.10^7$	$1.9.10^7$

St Georges: PLFA



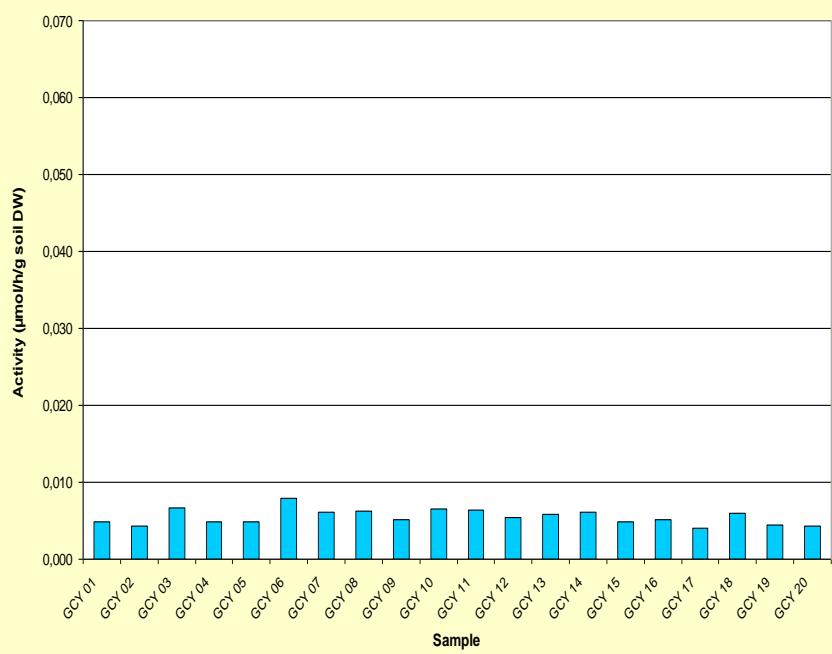
Yvetot: dehydrogenase

Meadow



30.0 ± 18.0 nmol/h/g soil DW

Crop: wheat



6.0 ± 1.0 nmol/h/g soil DW

- higher activity in the meadow, but with a higher spatial variation

Functional diversity: enzymes

Variable	St Georges meadow	St Georges crop	Yvetot meadow	Yvetot crop
Dehydrogenase (nmol/h/g soil)	11.0 ± 7.0	8.0 ± 5.0	30.0 ± 18.0	6.0 ± 1.0
Glucosidase (nmol/h/g soil)	266.1 ± 123.6	653.0 ± 162.6	670.0 ± 170.0	249.7 ± 76.2
N-acetyl gluco. (nmol/h/g soil)	486.2 ± 227.4	200.1 ± 81.1	212.0 ± 59.5	97.4 ± 72.4
Alk. phosphat. (nmol/h/g soil)	395.0 ± 90.3	506.5 ± 435.5	895.2 ± 642.8	747.8 ± 204.1
Aci. Phosphat. (nmol/h/g soil)	755.0 ± 354.3	640.0 ± 129.6	1368 ± 608	522.3 ± 321.9
Arylsulfatase (nmol/h/g soil)	91.0 ± 109.0	3.0 ± 48.0	444.0 ± 244.0	241.0 ± 10.0
Urease (nmol/h/g soil)	13.0 ± 4.0	10.0 ± 1.0	10.0 ± 4.0	8.0 ± 1.0

Potential functional diversity: Biolog plates

Yvetot

Crop

20 ▼ soil samples ► yes 1

156	0.985	1284	0.959	0.183	1.44	1721	0.613	0.063	-0.005	0.102	0.578	1709	1.001	1414	0.401	1829	1.078	0.037	0.708	0.23	0.314	0.047	1.28	0.008	-0.053	0.715	0.366	0.12	0.693	0.653
172	0.815	1.151	0.772	0.052	1524	1519	0.5	0.046	-0.003	0.049	0.327	1509	0.758	1258	0.295	1524	0.954	0.101	1937	0.343	0.27	0.109	0.097	0.007	-0.024	0.627	-0.004	0.117	0.434	0.773
1768	0.884	1244	0.73	0.122	1474	1819	0.486	0.031	0.025	0.064	0.391	1365	0.848	157	0.259	0.394	1.078	0.031	1852	0.312	0.363	0.009	1209	0.03	-0.038	0.951	-0.018	0.134	0.525	0.8
169	0.885	1045	0.824	0.217	1302	1555	0.634	0.083	0.16	0.044	0.606	1018	0.822	1.77	0.369	1767	0.772	0.041	189	0.299	0.486	0.029	144	0.015	-0.021	0.695	0.003	0.086	0.691	0.762
1712	1.006	0.922	0.877	0.057	1507	1498	0.643	0.095	-0.013	0.072	0.392	148	0.834	1491	0.304	1662	0.923	0.008	1861	0.216	0.234	0.097	127	0.022	-0.033	0.638	0.023	0.151	0.501	0.934
1782	1201	1717	1.126	0.151	1633	2.143	0.542	0.025	0.034	0.017	0.398	0.997	0.726	1.559	0.262	1908	0.942	0.006	1721	0.338	0.372	0.028	1764	0.012	-0.012	0.928	0.017	0.092	0.657	0.887
1372	113	1348	0.734	0.081	1352	1766	0.688	0.084	0.033	0.022	0.651	1754	0.857	1.27	0.443	1948	0.982	0.053	1984	0.399	0.277	0.05	1623	0.01	-0.037	0.657	-0.004	0.154	0.487	0.776
1858	0.904	1084	0.845	0.284	1457	1299	0.627	0.06	0.001	0.054	0.351	1569	0.903	1242	0.334	2.003	0.9	0.098	1801	0.124	0.315	0.1	1252	-0.009	0.004	0.537	0.025	0.066	0.937	0.725
1502	0.83	0.637	0.84	0.124	1454	1816	0.525	0.038	0.152	0.045	0.398	0.947	0.834	1.388	0.242	1768	0.932	-0.002	1816	0.287	0.355	-0.002	0.094	-0.01	-0.038	0.593	-0.049	0.1	0.49	0.909
1547	0.952	1044	0.969	0.058	1403	1767	0.616	0.047	-0.038	0	0.586	172	0.956	1441	0.423	188	0.958	0.068	1841	0.302	0.398	0.05	1485	-0.019	-0.074	0.851	0.365	0.142	0.508	0.678
1908	1315	1258	13	0.189	1664	1.198	0.729	0.11	-0.011	0.081	0.457	1441	0.776	1383	0.181	1867	1.102	0.108	1975	0.397	0.357	0.158	1087	0.286	-0.01	0.694	-0.007	0.162	0.405	0.779
1721	0.919	1031	0.715	0.104	134	1834	0.581	0.073	-0.013	0.009	0.446	1344	0.582	1.587	0.226	1867	0.917	-0.002	1784	0.202	0.365	0.011	0.786	-0.009	-0.042	0.685	-0.024	0.099	0.592	0.742
1844	0.942	1037	0.879	0.145	1463	198	0.624	0.18	-0.01	0.051	0.56	1573	0.614	1405	0.224	1749	0.703	0.085	1844	0.268	0.367	0.037	1525	0.001	-0.084	0.658	0.004	0.11	0.753	0.788
1921	1146	1748	1.089	0.329	1438	1898	0.684	0.18	-0.02	0.023	0.408	1524	0.697	1613	0.283	1837	0.918	0.032	1809	0.459	0.26	0.903	1147	0.021	-0.024	1049	-0.012	0.138	0.511	0.548
1541	0.818	1.19	1449	0.244	1447	1971	0.587	0.054	-0.008	0.082	0.373	1639	0.902	1638	0.342	2.021	0.875	0.032	1857	0.348	0.467	0.026	97	0.007	-0.042	1.04	-0.024	0.181	0.619	0.864
0.414	1076	801	0.297	0.286	1426	1991	0.743	0.162	0.031	0.047	0.593	1394	0.569	113	0.384	188	0.696	0.051	1251	0.419	0.467	0.084	1443	0.004	0.007	0.637	-0.016	0.136	0.43	0.862
1808	0.951	1087	0.335	0.23	153	1712	0.656	0.072	-0.028	0.038	0.553	0.791	0.474	1.386	0.426	2.084	0.661	0.052	2.023	0.206	0.397	0.05	0.797	0.062	-0.036	0.557	0.429	0.105	0.522	0.828
1778	0.847	975	0.28	0.257	1301	2.036	0.741	0.003	0.05	0.013	0.403	1432	0.352	1523	0.33	171	0.394	-0.002	1216	0.281	0.257	0.168	1644	0.001	-0.025	1.007	-0.024	0.159	0.359	0.87
0.643	0.919	1232	0.387	0.413	1382	1331	0.679	0.055	0.02	0.058	0.639	1354	0.836	1402	0.394	1843	0.876	0.036	127	0.236	0.379	0.106	1975	0.054	-0.032	0.76	0.001	0.113	1.075	0.984
2.004	0.941	903	0.087	0.28	1.196	1667	0.486	0.034	0.016	0.036	0.469	0.725	0.542	1.278	0.251	1606	0.575	0.017	1839	0.143	0.551	0.009	0.817	0.067	-0.032	0.696	0	0.135	0.452	0.83

Meadow

1696	1295	1663	0.772	0.715	1.341	2.268	0.819	0.215	0.012	0.113	0.813	1823	0.986	1.59	1.083	1966	0.969	0.262	1728	0.792	0.931	0.203	1952	1.068	0.02	1.455	1.589	0.214	0.518	0.1018
1748	1308	1219	1.075	0.352	1.19	1853	0.778	0.193	-0.004	0.041	0.45	182	0.926	176	1213	194	1.11	0.086	188	0.666	0.78	0.13	18	0.985	-0.049	1.194	1.764	0.155	0.646	1.068
1588	1356	1011	0.855	0.347	1.192	1925	0.6	0.155	0.009	0.013	0.414	1692	0.904	1332	0.98	1804	0.968	0.037	1546	0.569	0.6	0.025	1819	0.969	-0.05	1.137	1.533	0.197	0.383	0.806
1663	1403	1036	1.005	0.335	1326	2.337	0.758	0.221	-0.01	0.057	0.696	1945	0.951	1.737	0.47	1972	1.389	0.191	1899	0.798	0.596	0.107	2.047	1.087	-0.023	0.845	1.929	0.229	0.701	1.103
1694	1511	125	1.117	0.686	1436	2.084	0.765	0.171	-0.031	0.054	0.57	1878	0.984	2.014	0.189	1891	1.153	0.132	1787	0.65	0.548	0.023	2.081	1.18	-0.052	1.366	1.762	0.209	0.724	1.004
1902	1639	1492	1.54	0.621	1473	2.251	0.71	0.271	-0.031	0.02	0.634	170	1.195	1.495	1.232	1931	1.488	0.167	1517	0.662	0.837	0.116	2.013	1.015	-0.024	1.91	1.822	0.368	0.75	0.873
1709	1311	1388	0.86	0.51	1255	2.142	0.704	0.194	0.006	0.032	0.741	179	1.237	1.727	0.532	1931	0.954	0.151	1738	0.794	1.33	0.263	1805	1.072	0.006	1.475	1.831	0.239	0.574	1.017
1611	1593	1286	1.104	0.414	1302	2.042	0.751	0.288	-0.007	0.049	0.438	1689	0.946	1847	1.066	2	1.15	0.108	1827	0.731	0.615	0.21	1682	1.08	-0.024	1.098	1.768	0.188	0.514	1.025
1516	1276	0.948	0.945	0.259	1344	2.186	0.508	0.305	0.002	0.032	0.446	1405	0.804	1465	0.339	1779	0.906	0.019	1501	0.771	0.498	0.036	1987	0.982	-0.034	1.221	1.649	0.239	0.717	0.757
1511	1365	1657	0.876	0.577	1314	2.042	0.638	0.229	0.17	0.07	0.535	1671	1.048	2.077	0.671	1759	1.308	0.306	2.065	0.35	0.708	0.252	1924	0.95	0.061	0.981	1.912	0.273	1.071	0.921
1574	1566	0.845	1044	0.599	1374	1808	0.576	0.167	0.02	0.027	0.472	1937	0.586	1594	0.294	1849	1.174	0.167	1691	0.505	0.53	0.079	0.856	0.899	-0.026	0.712	1809	0.177	0.437	0.939
1754	1563	0.798	1063	0.399	1524	1956	0.649	0.205	-0.032	0.268	0.724	1563	0.95	1598	0.194	1908	1.25	0.023	1858	0.528	0.534	0.025	2.059	1.083	-0.025	0.583	1.656	0.179	0.603	0.934
1599	1492	0.985	0.827	0.815	1538	1948	0.664	0.238	0.007	0.062	0.479	1507	1.082	0.388	1.665	1.195	1704	0.623	0.7	0.209	1665	1.01	0.003	0.796	1432	0.235	0.509	0.889		
1405	1387	1267	0.999	0.8	1289	2.075	0.707	0.159	0.027	0.098	0.482	1522	1.042	1807	0.329	1745	1.14	0.133	1935	0.634	0.433	0.131	0.921	0.881	0.022	1.264	1.52	0.21	0.938	0.937
1634	132	1245	1	0.779	1305	2.164	0.72	0.196	-0.012	0.043	0.489	1588	0.802	1362	0.305	1807	1.175	0.125	1801	0.638	0.658	0.045	183	0.962	-0.019	148	1.357	0.183	0.902	0.755
1233	126	0.845	0.794	0.483	158	1832	0.645	0.115	-0.015	0.036	0.629	1755	0.874	1701	0.381	1893	1.04	0.051	1804	0.472	0.544	0.026	1478	1.052	0.016	0.778	1854	0.258	0.627	1.022
1552	1402	0.913	0.947	0.423	122	1982	0.495	0.149	-0.015	0.021	0.38																			

Conclusions

Structural diversity

- The mean values of the descriptors are higher in meadows than in crops
- Their spacial variations are lesser in crops than in meadows
 - ▶ meadows seem to exhibit high amounts of fungal/bacterial biomass

Functional diversity

- Meadows strongly differ from crops
- The diversity of bacteria present in meadows is higher than this measured in crops
 - ▶ meadows seem to exhibit the highest functional diversity
 - ▶ Decreased selectivity of functional variables vs diversity

Perspectives

- Variation of the descriptors with the season
- Profiles of soil enzymes
- Relationship with physico-chemical descriptors
- Impact of pollutant
- Database
- Mathematical treatment
- Development of new descriptors

Flow cytometry

5 g + 45 ml NaCl



Vortex, centrifugation 1000 rpm 5mn



Supernatant, dilution in NaCl

10^{-1} et 10^{-2}

10^{-2} et 10^{-3}

**Labeling with SYBR Green I
0.05 % final (488-525 nm)**

Cytometry (13 µL)



Quantification of total bacteria

**Spreading on R2A medium: 100 µL
Incubation**



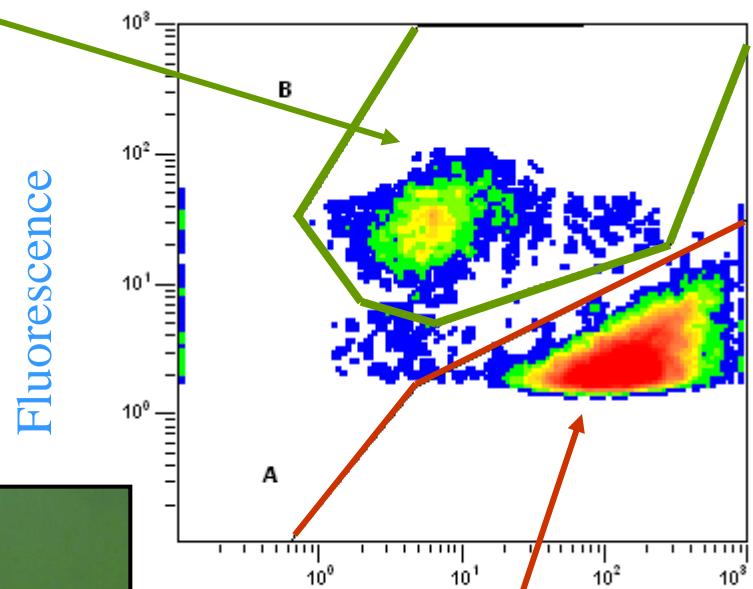
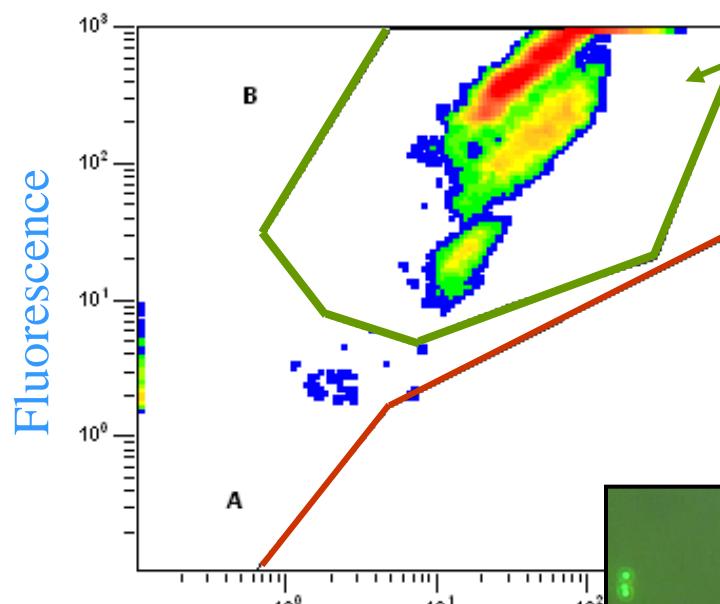
Enumeration of cultivable bacteria

Cytograms

Pure strain of
Pseudomonas from soil

Soil

Bacteria



Soil particle

Effect of xenobiotics on the production of laccase proteins

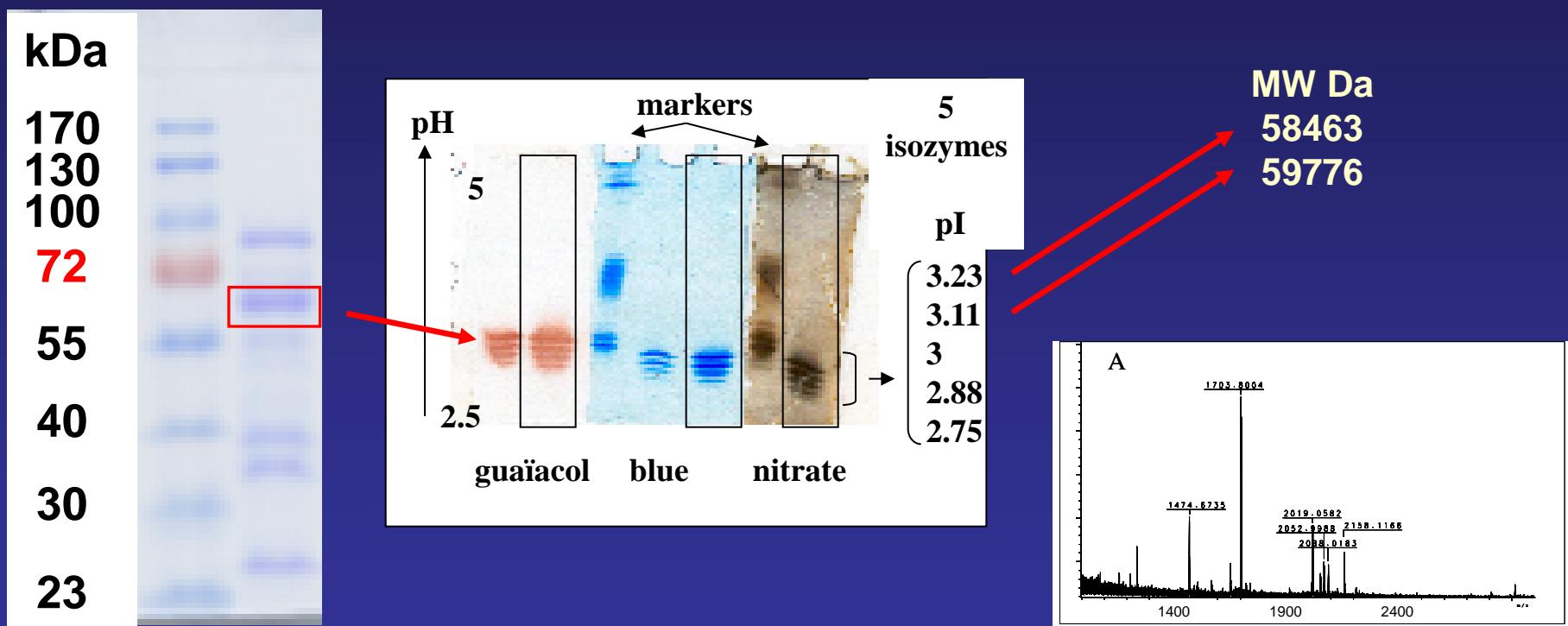
- Treatment of 4-day liquid cultures of *T. versicolor* with xenobiotics
- Separation of the extracellular fluid
- Precipitation of polysaccharidic compounds with 10% acetone
- Diafiltration of the protein solution (cut off 10kD, Amicon)
- Purification on Concanavalin A-Sepharose 4B column (Sigma-Aldrich)
(elution with 0.2M Tris HCl in the presence 0.5M NaCl and 0.2M o-methyl glucose)
- Separation by SDS-Page (12%)
- Identification of proteins by Maldi-TOF (Bruker)



profiles of proteins

Separation and identification of lac A isoforms

1st migration
mass → 2nd migration
pl → Maldi-TOF



► Towards a proteomic approach of soil diversity

Partnership

Soil biological descriptors

BioSol – ESITPA

M. Akpa, C. Bailleul, S. Desaire, C. Gangneux, I. Gattin,, K. Laval, M. Legras

Phytopharmacy – INRA

A. Brault, N. Demont, J. Dubroca, J.C. Dur, C. Jolivalt, C. Malosse, C. Mougin

LMDF – University of Rouen

S. Baray, N. Cariou, B. Pawlak, P. Plassart

Soil physico-chemical descriptors

Soil Science Unit – INRA

E. Huard, J.P. Leydecker, M. Pernes, D. Tessier, N. Wolf

Mathematical modelling and biomathematics

LAMSAD – ESITPA

S. Taibi

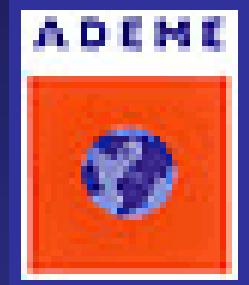
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A.S. Lemaire

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www.esitpa.org/biosol

www-phyto.versailles.inra.fr