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Resilience of the rhizosphere to anthropogenic disturbance

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Abstract

The rhizosphere is a dominant site of microbial metabolism in soil and whereas it can be shown that anthropogenic disturbances can influence this metabolism, the impact of these disturbances on biodiversity is rather difficult to determine at the species level. This is in part because no more than 10% of the microbial species are culturable, and in part because there is very poor precision in plate counting, usually requiring a change of 300–500% to be significant. We have therefore used a functional approach. The 'ecophysiological index' is based on *r* or *K* strategy of the organisms being counted. Also, enzyme families, microbial biomass, microbe/microbe and microbe/fauna interactions have been determined, along with nutrient uptake measurements. The techniques have been applied to determine the effects of disturbances created by the introduction of GM plants and microorganisms to soil, these effects being small compared with those caused by time-honoured practices such as crop rotation and ploughing. Toxicity from industrial influences (e.g. cyanide) can be remediated by rhizosphere microorganisms.

Introduction

In environmental impact issues it is normally useful to start with a definition of terms to be discussed. In the present context, it is reasonable to consider the conference Soil Resilience and Sustainable Land Use (Greenland & Szabolcs 1994). There *soil resilience* was defined as the ability of the soil to recover after disturbance. A related term is *soil quality* which is the capacity of the soil to produce healthy and nutritious crops, resist erosion, and reduce the impact of environmental stresses on plants (Papendick & Parr 1992). The Oxford English Dictionary indicates that *anthropogenic* is 'originated by man'. There is, thus, scope for a wide range of factors concerning agricultural practice, industrial activity and biological modification which can be regarded as anthropogenic (Table 1). In this mini review, the examples of agricultural practice which will be discussed are ploughing and crop rotation. It could have been equally relevant to have considered crop protection and enhancement, where there has been concern for many years on the influence of fertilisers and crop protection agents on the ecosystem. An alternative to pesticides is biocontrol; when this is practised augmentatively, as opposed to allowing natural actions without encouragement, it can again be regarded as anthropogenic. In recent years, however, there has been interest in not using simple augmentation but to genetically modify the organisms prior to their addition to the ecosystem, in essence a 'double anthropogenesis'! This will be the focus of the example on biological modification. Cross-infection could well have been discussed because man can often be the vector of pests and diseases. This is of course not just of crops, but also of animals and man. We have seen in 2001 in the UK the dire consequences of the transport of animals around the country resulting in a rapid spread of the foot and mouth virus.

There are multiple ways in which industrial activity can pollute the soil, aquatic and atmospheric ecosystems. Factory effluents, gasworks and power cables can all leach pollutants to the terrestrial environment while mining activities can produce noxious residues after ore extraction. Cyanides are good illustrations of effluents and will form the example of industrial pollution in this discussion.

The rhizosphere is the field of action around, on and within a root (Lynch 1990; Pinton et al. 2001).

Table 1. Examples of anthropogenic disturbance.

Agricultural practice	Biological modification	Industrial pollution
• Ploughing	• Biocontrol	• Brownfields
• Crop rotation	• GM crops	• Mine tailings
• Irrigation	• Cross-infection	• Gaseous emissions and climate change
• Fertilization		
• Liming		
• Crop protection and enhancement		

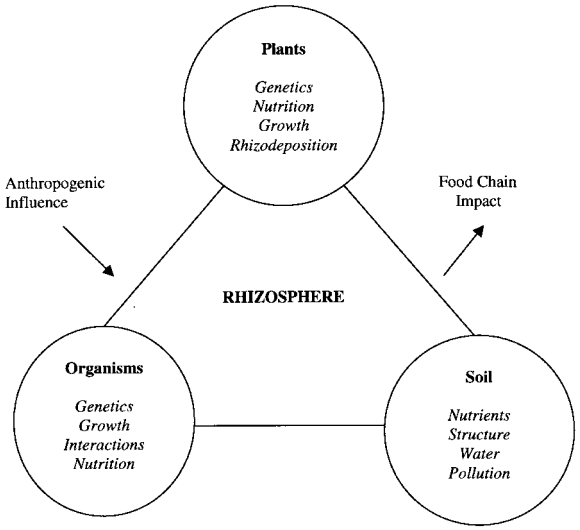

Figure 1. The rhizosphere trinity.

It is thus a trinity formed by plants, organisms (microbes and fauna) and soil (Figure 1). The rhizosphere is where up to 40% of the photosynthate captured ends up as rhizodeposition products, most of which is available to soil organisms (Whipps & Lynch 1983).

Over the past decade or so, it has become increasingly recognised that the interactions between the root and organisms are under genetic control, including the recognition, regulation and expression of pathways which lead to beneficial, harmful or neutral effects on the soil ecosystem. As the soil's energy powerhouse therefore, it is crucial for sustainability that the rhizosphere be resilient to anthropogenic disturbance. In order to determine the resilience, a variety of monitoring options are available.

Traditionally microbial populations have been used as indicators of change. However it is rarely possible to count soil organisms, even on selective media, to give an error less than 0.5 1og units (a 300% change), which many would regard as a catastrophic change on an ecosystem. An improvement is to measure colony development on isolation plates with time (De Leij et al. 1994). Counts are made for each of ten days and the number of colonies in each daily group assessed as a population and the numbers fitted to an ecophysiological index (H′), which is mathematically the same as the Shannon index of diversity.

$$H' = -(\mathrm{Pi} \times \log_{10} \mathrm{Pi})$$

where Pi = population in class/total population. For even distributions $H'_{\max} = 0.826$. For even distributions $H'_{\min} = 0$.

This index is a more sensitive indicator of perturbation and has more ecological significance than routine colony counting at one moment in time. For example Table 2 compares the H^1 indices in the rhizosphere of wheat grown in the field and in glasshouse microcosms (De Leij et al. 1994). It can be seen that at flowering the field soil has a lower EP index than the soil microcosm, whereas the situation reverses at the root surface. However, the problem with all counting techniques is that probably no more than 10% of species are culturable and so a totally false impression might be gained.

Table 2. EP-indices for bacterial populations in soil and on the root surface of spring wheat (after De Leij et al. 1994).

	Soil		Roots	
Growth stage	Flowering	Ripening	Flowering	Ripening
Field	0.675	0.672	0.619	0.610
Glasshouse	0.710	0.646	0.427	0.611

SED 0.022 Fprob**.

Routinely now it is possible to measure microbial genes rather than populations. Techniques such as ARDRA (amplifed ribosomal DNA restriction analysis) and RAPD (analysis of randomly amplifed polymorphic DNA markers) facilitate molecular taxonomy as grouping of isolates and molecular taxonomy for strain identification respectively. Such analyses are not dependent on the culturability of organism unless they are applied on isolates. These, however, have limited uses in population studies and whereas they give an insight into the presence of genes in an ecosystem, they do not give any real indication of gene expression, which is ultimately what determines environmental impact. However mRNA can be measured for specific microbial genes from soil, thus providing specific information on gene expression. DNA microassays should facilitate the measurement of a range of functions at the mRNA level. Enzyme activity can be readily measured in the rhizosphere with greater precision than population counting and can be particularly useful when suites of enzymes are measured (Naseby & Lynch 1997). A problem however is that the source of the enzyme is not usually identified. Furthermore only potential activity is measured. This can be overcome by measuring the enzyme product, namely the accumulated pool of a biological reaction in soil, by standard chemical methods which are much more precise than biological methods. The downside on this is that there is seldom scope to distinguish if those pools in the soil originate from biological reactions or anthropogenic sources.

Ultimately, however, the question is whether anthropogenic influences affect the function of organisms in the environment and, in this respect, bioassays are a useful tool in ecotoxicology. This can be as simple as a seed germination test or testing the effect on aquatic organisms. In terms of impact on man, more appropriately described as environmental toxicology, cell cultures (for example the Ames test) or microbial whole-cell biosensors (using amperometric signals or reporter genes), can be applied to soil samples.

Ploughing, fertilisation and crop rotation

One of the first influences that man had on natural ecosystems to create agriculture was to plough the soil and to rotate crops. Both of these influences are massive as illustrated by the measurement of soil biomass using the fumigation-respiration technique of Jenkinson & Powlson (1976). In sequential measurements through the year it was found that, as roots grow, the soil biomass increases to a maximum in time, coinciding with the time of maximum root production (Table 3). Also, when crops seeded in ploughed soil were compared with direct-drilled crops, the biomass in the surface layer (5 cm deep) was far greater in the direct-drilled soil, corresponding with a proportionate increase in rooting in those layers. This is a very clear demonstration of the rhizosphere affect. Recently in a study in Denmark using a similar biomass estimation technique, it was demonstrated that the rhizosphere effect on biomass extends 2.5 mm from the root surface in fertilised soil, but to only 1mm in infertile soil (Neergaard & Magid 2001). By adding ^{14}C labelled glucose into the soil, it was found that microbial ^{14}C increased threefold near the roots in the fertile soil as a result of assimilation of previously formed microbial residues, but in the infertile soil there was no increase. Therefore, *inter alia* it can be assumed that fertilisation influences rhizosphere biomass greatly.

The rhizosphere effect can also be investigated by comparing rooting and biomass in a soil carrying an annual crop with a soil in a perennial crop. It can be seen that a grassland has a far greater rooting density in its surface 5 cm than the crop carrying oil-seed rape and this leads to a much greater microbial biomass (Table 4). Interestingly the soil organic matter is significantly greater with grassland but not to the same extent as reflected by the root and microbial biomass. This is because the majority of soil organic matter is humic material which has been ^{14}C dated to have a half-life in excess of 1,000 years (Campbell et al. 1967). What this all implies, however, is that the cropping pattern, including rotations and grass leys, will have a profound influence on the size of the rhizosphere biomass. It should also be considered that the organic rhizodeposition products from different plant species vary greatly (Whipps & Lynch 1986) and therefore it can be expected that the structure and composition of the rhizosphere biomass will vary greatly with the influence of crop rotation.

Biocontrol/genetically modified organisms

The principle purpose of biocontrol of root diseases is to change the rhizosphere to eliminate harmful and pathogenic organisms. It could also be considered as a normalisation practice to generate sustainable crop productivity without the adverse effect of pests and

Table 3. Biomass of ploughed and direct-drilled clay soil planted with winter wheat (after Lynch & Panting 1980).

Biomass (KgC ha^{-1})	August	November	April	June	July	August
Direct drilled	286	178	200	507	452	400
Ploughed	234	178	139	430	391	200

Table 4. Estimates of soil biomass in a clay soil under grassland or oil-seed rape (after Lynch & Panting 1982).

	Root weight (mg dry wt g^{-1} dry soil)	Soil organic matter (% w/w)	Microbial biomass (KgC ha^{-1})
Grassland	12.0	4.5	1060
Oil-seed rape	0.4	4.1	370

Table 5. Effect of metabolic load on the competitive ability of genetically marked *Pseudomonas fluorescens* strains in the rhizosphere (after De Leij et al. 1998).

Metabolic load	Percentage of total population		
	Day 0	Day 14	Day 28
aph 1	30	52	55
alp 1, *xylE*	45	23	20
aph 1, *xylE, lacZY*	25	25	25

diseases. In any event, an anthropogenic perturbation is intentional. The perturbations can be reduced by encouraging intrinsic organisms to proliferate or by adding exotic organisms (bioaugmentation). The problem comes if other unintentional harmful side-effects occur. With crop-protection chemicals, the aim has been to determine these effects but there has been less concern with biocontrol agents. That is until molecular biologists started to try to improve these by genetic modification. For example most of the soya crop now produced in North America is genetically modified. All manner of scare stories were relayed to the public, including the concept of the generation of "Frankenstein Foods", a term coined by action groups, by gene flow. We initially investigated this by carrying out the first release of a genetically bacterium in the UK (De Leij et al. 1995a, b; Thompson et al. 1995). Modification of *Pseudomonas fluorescens* SBW25 was with the marker genes *lacZY* and *xylE* linked to aph1 (kanamycin resistance) spatially separated on the chromosome. There was no horizontal transfer of these chromosomal inserts and, not surprisingly as there were no functional genes inserted, there was little effect on rhizosphere populations. However, in a further study it was seen that the insertion of the genes placed a metabolic burden on the bacterial host cell and the ecological competence of the introduced bacteria was reduced (Table 5). This burden, although reducing fitness and some aspects of value, can be a valuable biosafety vent.

In order to investigate the effect of functional genes in the rhizosphere two bacteria were used *P. fluorescens* F113 which is a wild-type producing the antifungal antibiotic diacetylphloroglucinol (DAPG), and *P. fluorescens* F113 G22 which is modified to delete DAPG production (Fenton et al. 1992).

In the wheat rhizosphere, alkaline phosphatase activity was increased, while chitobiosidase, aryl sulphatase and urease activities were decreased by DAPG production (Naseby & Lynch 1997). In the pea rhizosphere, alkaline phosphatase, aryl sulphatase and urease were increased and β-glucosidase and N-acetylglucosaminidase were decreased by the introduction of the DAPG-producing organism (Naseby & Lynch 2001). The effects of the DAPG-producing strain on populations were to shift indigenous bacteria from *r* to *K* strategy (Naseby & Lynch 1998), enhance nodulation (Table 6; Andrade et al. 1998), reduce the total culturable bacterial population (Naseby & Lynch 1999), and increase rhizosphere bacterial and fungi (Naseby & Lynch 2001). DAPG stimulated an increase in nematode numbers in the pea rhizosphere but decreased nematode populations in the wheat rhizosphere. As faunal grazing leads to increased mineralisation of microbial N, and therefore N increased in pea inoculated with the DAPG producer (Table 7). The anthropogenic influence was to introduce and elevate the wild-type population of the bacterium in the rhizosphere, but the further influence was the deletion of the antibiotic production gene. In-

Table 6. Effect on *Rhizobium leguminosarum* 1112 and *Pseudomonas fluorescens* F113 and F113G22 on nodulation and pea plant growth (after Andrade et al. 1998).

Treatment	Shoot dry weight (g)	Number of nodules per g root
Control	1.41^{d}	5.1^{a}
R. leguminosarum 1112	1.24^{cd}	7.9^{ab}
P. fluorescens F113	1.02^{abc}	9.9^{b}
1112 + F113	0.89^{ab}	20.3^{c}
P. fluorescens F113G22	0.81^{a}	7.9^{ab}
1112 + F113G22	1.22^{bcd}	6.0^{ab}

ures not followed by the same letter are significantly different ($P = 0.05$)

	Pseudomonas fluorescens strains
F113	Wild type which produces DAPG
F113 G22	Modified to delete DAPG production

Table 7. Nematode and nitrogen uptake of plants in response to bacterial inoculation (after Brimecombe et al. 2000).

	Nematodes g^{-1} soil	% N derived from organic residue shoot
Pea		
Control	2.9^{a}	8.3^{a}
F113 G22	4.9^{ab}	20.3^{b}
F113	6.2^{b}	25.7^{b}
p	*	**
Wheat		
Control	4.5^{a}	29.4^{b}
F113 G22	3.3^{b}	20.7^{a}
F113	2.6^{b}	22.7^{a}
P	**	*

Table 8. Effect of *Pseudomonas fluorescens* strains on *Pythium* infected plants (after Naseby et al. 2001).

Strain	Shoot Weight	Root weight	Root length	Number of lateral roots
F113 (DAPG)	+20			
SBW 25 (competitive exclusion)	+22	+14	+19	+14
CHAO (HCN)	+35	+52	+69	+29
Q2-87 (phenazine)	+14			

All figures are expressed as a percentage increase relative to control infected plants where there is a significant effect at $P = 0.05$.

terestingly, in comparing various *P. fluorescens* strains with the capacity to produce a range of antifungal compounds, the wild-type SBW 25 strain used in the marker studies and which acts by competitive exclusion proved to be an excellent agent to control *Pythium* in peas (Table 8). The strain CHAO which produces hydrogen cyanide (Duffy & Defago 1997) was also very useful. Interestingly, however, it has been suggested that other beneficial rhizobacteria (or plant growth promoting rhizobacteria, PGPRs) act by catabolising cyanide (Bakker & Schippers 1987). The phenazine producing strain Q2-87 (Mazzola et al. 1997), which has been used extensively for biocontrol, was not so effective, giving a small root weight increase as its only significant effect.

Cyanide

Cyanides are common contaminants of brownfield sites, especially old gasworks. They are also present in effluents from gold mines. In 2000 the media reported widely that a cyanide-tainted waste spill from an Australian-owned mine in Romania caused up to 80 per cent of the fish to be killed in the River Tizsa, which runs through Yugoslavia and Hungary as well. More worrying was that water supplies for more than 2.5 million people were threatened by the discharge of 378,500 lites of cyanide-contaminated water from the reservoir at the Aural Gold Mine at Baia Mare. Frequently the cyanide forms a complex with metals. Barclay et al. (1998) have demonstrated that *Fusarium solani* and *F. oxysporum* has the capacity to bioremediate especially the metallocyanides. Unfortunately many strains of *Fusarium* can be pathogenic in the rhizosphere. We have shown that even though cyanide is naturally formed in low concentrations in the rhizosphere (Dartnell & Burns, 1987) concentrations around 10 mM are very phytotoxic (M. Ezzi & J.M. Lynch, unpublished). These concentrations of free cyanide can be rapidly catabolised by selected strains of *Trichoderma* in rhizosphere soil through the action of formamide hydrolyase and rhodanase (Table 9). Thus the anthropogenic actions of cyanide introduction into the soil leading to phytotoxicity and even human tox-

Table 9. Trichoderma cyanide catabolism (M. Ezzi & J.M. Lynch, unpublished).

Strain	Enzyme (μmoles/hr/mg protein)	
	Formamide hydrolyase	Rhodanase
10	5.5	0.30
TH1	5.5	0.59
WT	2.9	0.91
T12	4.7	0.89

Formamide hydrolyase

$$HCN + H_2O \rightarrow HCONH_2$$
(plants and fungi)

Rhodanase (thiosulphate: cyanide sulphur transferase)

$$S_2O_3^{2-} + CN^- \rightarrow SO_3^{2-} + SCN^-$$
(animals and *Pseudomonas*)

β-cyanoalanine synthase (L-cysteine hydrogen sulphide-lyase)

$$HSCH_2CHNH_2H + HCN \rightarrow NCCH_2CHNH_2C0_2H + H_2S$$
(plants and *Enterobacter*)

Figure 2. Cyanide catabolizing enzymes.

icity, can potentially be remediated by the introduction of cyanide-catabolising micro-organisms.

Conclusions

The rhizosphere is the energy powerhouse of the plant/soil ecosystem, leading to the sustainability of agriculture and forestry. The resilience of the system can be diminished by toxic insults and even conventional agricultural practices. The following points need consideration in the protection of the system.

- Monitoring methods to assess changes are improving but need further development.
- Established agricultural practices can have profound influence on the rhizosphere ecosystem which is seldom recognised.
- Industrial pollution needs to be assessed as it can be very toxic, but potentially can be bioremediated.
- Pests and diseases are ever-present threats and cross-infection of plants and animals can easily occur by a variety of anthropogenic transmissions.
- Generally GM effects on the ecosystem have been very small.

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