ROTHAMSTED RESEARCH

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Environment and Nutrient Interactions

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Environment and Nutrients

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Increasing the efficiency of uptake of nutrients by crops is a key objective for agricultural research worldwide. Studies on the molecular basis of nitrate and sulphate uptake by roots are providing fundamental understanding that will be used in the genetic manipulation of plants for improved nutrient utilisation. The dynamics of nutrient pools within plants are being studied as the basis for diagnostic measurements for monitoring nutrient status during a growing season and fine-tuning fertiliser applications. Soil microorganisms mediate the processes that determine nutrient supply to crops. Their activities also determine a range of ecosystem functions. Research to characterise the activity and diversity of soil populations is directed towards the development of biological indicators of soil quality.

MINERAL NUTRITION

Several research programmes within the Institute are directed towards finding ways of increasing the efficiency of fertiliser use, so that inputs may be reduced without significant loss of yield or quality. This not only reduces costs for the farmer but also helps to minimise the undesirable impacts that nutrient losses from the soil can have on the environment. One approach is to investigate the mechanisms that plants use to acquire their nutrients from the soil, with the eventual aim of using genetic manipulation to improve the efficiency of nutrient capture. Another is to identify ways of diagnosing a plant's nutrient status so that fertiliser applications can be tailored more accurately to meet the needs of the crop.

The uptake of nutrients from the soil solution into root cells is a complex process involving many different protein components (transporters) located in the plasma membrane surrounding the cell. In the past decade there has been remarkable progress in our understanding of these transporters at the molecular level, and work within the Institute has made a significant contribution to this, particularly with respect to the uptake systems for nitrate and sulphate.

Molecular biology of nitrate and sulphate uptake

In agricultural soils nitrate is the main source of N available for crop growth. The process of nitrate uptake from the soil requires energy and is known to be mediated by at least three different transport systems. We have identified one gene family (the *NRT2* family) which has been found in all plant species so far analysed and which is closely related to genes for nitrate transporters in fungi and algae. *Arabidopsis thaliana* (a model plant species which is a member of the mustard family) has two *NRT2* genes, but only one of these appears to be active in roots and its expression is only switched on when nitrate is present.

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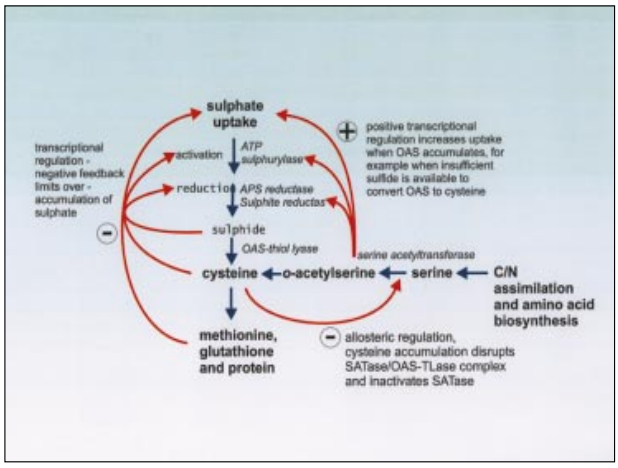


Fig. 35 *Metabolic control of sulphate uptake and assimilation*

To find out what role the *NRT2* gene product plays in nitrate uptake we have generated transgenic *Arabidopsis* plants in which the expression of the *NRT2* gene is strongly suppressed. The resultant transgenic lines were found to have lost about 40% of their capacity for nitrate uptake from low concentrations of nitrate (< 0.25 mM). This indicates that the *NRT2* gene product is an important component of the nitrate uptake system in *Arabidopsis* roots.

Recent work has led to the cloning of multiple genes for sulphate transporters, including three from the tropical legume, *Stylosanthes hamata*, one from barley, and two from the diploid wheat, *Triticum tauschii*. An analysis of these sequences and a homologous family of seven transporters from *Arabidopsis* clearly demonstrates that sulphate transporters fall into several sub-groups. It is thought likely that each sub-group is responsible for different steps in the movement of sulphate around the plant, but their exact functions have yet to be defined.

Before we can attempt to manipulate sulphate transport and storage within the plant we need to understand how the various pools of sulphur respond to changes in S availability. Our studies have revealed that different crop species can respond very differently. In mature leaves of oil-seed rape, for example, reserves of sulphate can accumulate when supply is in excess, but these reserves are not readily available to the younger parts of the plant when they need it. The remobilisation process in oilseed rape is therefore a clear target for genetic manipulation. By contrast, sulphate reserves in wheat leaves are readily mobilised to the developing ear, but accumulation of these reserves only occurs when there is substantial excess available in the soil. In this instance the most promising targets for manipulation would seem to be the regulatory mechanisms that limit sulphate uptake.

Brian Forde and Malcolm Hawkesford - (IACR-Rothamsted)

Regulation of nitrate and sulphate uptake

The uptake systems for both nitrate and sulphate are highly regulated. In both cases there are feedback mechanisms that control the rate of ion uptake according to the plant's requirements, preventing over-accumulation of the respective nutrient. At present very little is known about the mechanisms by which the plant monitors the external availability of a nutrient and its internal nutrient status and integrates this information to regulate nutrient uptake activity. An understanding of these processes will be important for future attempts to genetically manipulate the uptake systems to increase the efficiency of nutrient acquisition.

The expression of sulphate transporter genes is highest under conditions of S deficiency, so maximising the plant's capacity for sulphate uptake. Research in the Institute has shown how this control is mediated by metabolites of the S-assimilatory pathway (Fig. 35). Transporter gene expression is repressed by the end-products of sulphate assimilation and is activated by the precursor O-acetylserine (OAS), which serves to co-ordinate S-assimilation with C and N metabolism.

In the case of nitrate, the relevant transporters are similarly subject to feedback repression from the products of nitrate assimilation but, unlike the sulphate transporters, they are also induced by the presence of their substrate (nitrate). There is some debate as to where the presence of nitrate is sensed: do root cells monitor the external nitrate concentration directly (through

Fig. 36 *A hand-held SPAD meter*

an externally located nitrate sensor) or indirectly (through changes in the cytosolic nitrate concentration)? Studies using nitrate-selective microelectrodes to measure the nitrate concentration in the cytosol and the vacuole of individual barley root cells have thrown some light on this. It was found that even ten thousand-fold changes in the external nitrate concentration had little effect on the nitrate concentration in the cytosol of mature root cells, while the nitrate concentration in the vacuole was more closely related to the external supply. Only when seedlings were starved of nitrate for more than two days and vacuolar nitrate was depleted did the mean cytosolic nitrate activity significantly decrease.

Thus in fully developed vacuolate cells of the root there appears to be a regulatory mechanism that maintains the cytosolic nitrate concentration within tight limits. This might suggest the need for an external nitrate sensor. At the root tip, however, where cells have not yet developed a vacuolar nitrate store that can be used to buffer the cytosolic pool, the cytosolic nitrate level is more responsive to external fluctuations in nitrate availability . Thus in these cells it is possible that an extracellular nitrate sensor would not be required and changes in nitrate availability could be monitored through fluctuations that occur in the cytosol.

Tony Miller - (IACR-Rothamsted)

Diagnosing crop nutrient status

Reliable diagnosis of the nutrient status of a crop is far from simple. Practical diagnostic indicators must be sensitive, stable, specific and easily measured. The most commonly used indicator for plant N status is total-N expressed as a percentage of shoot dry matter. However, critical concentrations for growth and yield expressed in this way decline with crop growth stage, are affected by growth conditions and are not easy to measure. Some of these effects can be reduced by targeting organs of a fixed physiological age (e.g. the last fully expanded leaf), by targeting specific N pools (e.g. the nitrate storage pool), and by expressing concentrations on a tissue water or leaf area basis. These approaches are currently being investigated for winter wheat.

An alternative approach is to use a so-called 'tracker' pool. Leaf greenness has long been used as a crude qualitative indicator of plant N status; leaves vary from pale yellow to blue-green depending on N supply (and other factors). Chlorophyll, the pigment responsible for leaf greenness, is an important but minor N pool which amounts to about 2% of total leaf N. There has been a recent resurgence of interest in chlorophyll as a tracker for total-N due to the ease with which it can now be measured either in single leaves with a small hand-held spectrometer (Minolta SPAD-502 meter) (Fig. 36) or remotely in canopies with a spectroradiometer.

SPAD meter readings are well correlated with leaf chlorophyll and nitrogen in winter wheat. The correlations were not improved by expressing concentrations on a leaf area basis. SPAD meter readings on the last fully expanded leaf at growth stage (GS) 37 are also well correlated with grain yield (Fig. 37). In this particular trial, maximum grain yield was obtained with a meter reading of 48 at GS 37. Clearly this meter has the potential to be used for fine-tuning fertiliser applications during the season. The problem with using the meter or any indicator method to predict future N requirements is that the critical value is likely to be dependant on site and weather conditions. Results over several seasons with the wheat variety Hereward have produced a critical SPAD range of 48-53 at GS 37 for maximum yield. Work is continuing on the effects of environmental variables (other than N supply) on relationships between meter readings, leaf chlorophyll, leaf N and the growth and yield of wheat.

Until recently crops in the UK benefited from an abundant supply of free S from the air (derived from atmospheric pollution), so there was little need for farmers to apply S fertilisers. Now that there is much less S in the atmosphere, crops in some parts of the country are at increasing risk of S-deficiency. An H-GCA-sponsored project is evaluating the usefulness of metabolite pools containing S as early diagnostic indicators of S-deficiency. Sulphate is one of the most abundant pools of S in mature leaves

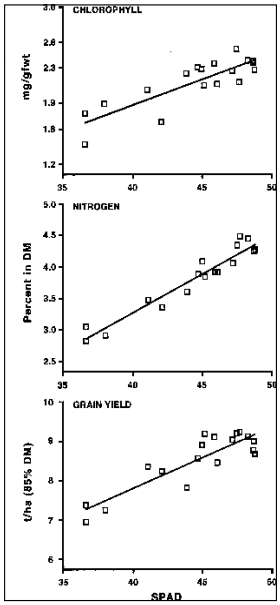


Fig. 37 *Relationships between SPAD meter readings at GS 37, chlorophyll and nitrogen contents in the last fully expanded leaf, and grain yield of winter wheat (cv. Hereward)*

of oilseed rape and was found to be highly responsive to the availability of S. However, use of the sulphate pool as a diagnostic aid would be complicated by the additional finding that this pool also fluctuates according to the stage of plant development.

Peter Barraclough - (IACR-Rothamsted)

Self-reporting of plant nutrient status

In an alternative approach to nutrient diagnostics, a project is underway to look at the feasibility of developing 'smart' plants which are able to report on their nutrient status through some easily detectable signal that is linked to subtle changes in gene expression. In the first stage of this programme we are seeking to identify genes that are switched on in leaves as part of the process of adaptation to reduced nutrient status. These could be genes involved in nutrient transport or in mobilising the plant's internal reserves of that nutrient. Using a technique called 'differential display' we are producing 'fingerprints' of the mRNA expression pattern in leaves of plants that have been subjected to the withdrawal of specific nutrients (N, P or S) for short periods of time. So far we have identified several dozen candidate mRNAs that increase in abundance within three days of withdrawing the N supply. Once it has been established which of these mRNAs is regulated in the appropriate way (i.e. responsive specifically to N status and not to changes in other nutrients), the promoters of the corresponding genes will be isolated. These promoters will then provide a genetic 'switch' that can be linked to a suitable reporter gene (such as green fluorescent protein) and reintroduced into the plant to provide an easily detectable marker that will allow the farmer to monitor the N status of the crop.

Brian Forde - (IACR-Rothamsted)

SOIL QUALITY

What is soil quality? Many farmers consider that they can easily recognise good soil quality but defining it in quantitative scientific terms has proved more difficult. Policy makers worldwide are demanding measurable indicators in order to formulate soil protection policies. Table 1 lists a set of 17 physical, chemical and biological properties that have been proposed as the minimum required for measuring soil quality[1]. In addition it is necessary to specify maximum concentrations of various toxic substances including metals such as cadmium and zinc and organic pollutants such as PCBs (Fig.38).

Table 1. Proposed minimum data set of physical, chemical and biological indicators for screening the condition, quality and health of soil

Physical:
- texture
- depth of soil, topsoil and rooting
- infiltration rate and bulk density
- water hold capacity

Chemical:
- soil organic matter
- pH
- electrical conductivity
- extractrable N, P and K

Biological:
- microbial biomass C and N
- potentially mineralisable N
- soil respiration

There is no simple, single measure. Soil quality is multi-functional and is best considered in relation to the use to which the soil is put. We therefore prefer to define soil quality in terms of fitness for use(s): soil should be in the proper physical, chemical and biological state for at least its present function and, preferably, for other functions in the future. For example, it may be desirable for arable farmland that becomes surplus to requirements to be converted to species-rich grassland or woodland.

Once we accept the definition of fitness for use we can usually identify a few factors that determine fitness for a specific use. In the case of nutrient status, for example, soil used for conventional arable agriculture must be able to supply 200, 20 and 200 kg ha^{-1} N, P and K, respectively, to crops each year; by contrast, plants growing on chalk downland recycle 10, 1 and 10 kg ha^{-1} of these same nutrients. With regard to pH, the optimum for arable land is 6.5-7.0 in water; chalk grassland may typically be at pH 8.5 and peat at less than 4.

In the UK, the Royal Commission on Environmental Pollution[2] suggested that the basis for soil resilience may well be the diversity, adaptability and shifting community structure of its microorganisms. They recommend that biological indicators of soil quality should be developed. This is not a simple task in view of the complexity and heterogeneity of soil populations which makes them more difficult to study than those in other habitats such as water. For example, the total bacterial population of a fertile agricultural soil may be at least 10^9 bacteria g^{-1} soil, although only 1-10% of these will grow on simple agar media under laboratory conditions. Little is known about the status of the unculturable organisms. Similarly, fundamental questions remain to be answered regarding the survival strategies adopted by soil organisms. The total population size, as determined by the microbial biomass methods developed at Rothamsted, is

Fig. 38 *Staff from the Soil Science Department, IACR-Rothamsted, sampling soil at an industrial site*

much larger than would be expected on the basis of energy inputs.

Techniques for studying the functions and diversity of soil populations, some of which are described below, can now provide data which were previously inaccessible. However, much remains to be learned regarding the appropriate interpretation of such data. We are seeking to develop the understanding required to apply these techniques in meaningful ways, with the aim of developing a suite of methods which may be combined to answer questions on soil quality relevant to a wide range of situations. Time can be saved by focusing on the weakest link in the chain of soil quality.

[1]Doran, J. et al. (1996). *Advances in Agronomy* **56**, 1.

[2]Royal Commission on Environmental Pollution 19th Report: *Sustainable use of soil.* London, HMSO, 1996.

Keith Goulding - (IACR-Rothamsted)

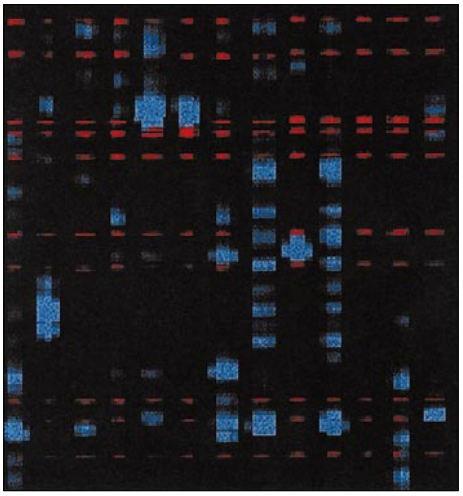
Genetic diversity of soil microorganisms

Certain soil microbes, in particular the 'autotrophic' bacteria which do not rely on organic carbon and nitrogen compounds for growth, and which include the nitrifying bacteria responsible for conversion of ammonia to nitrite and nitrate, and the methane-oxidizing bacteria, are difficult to culture in laboratory conditions. It is relatively difficult to assess their population size and little is known about their genetic diversity. In contrast, many heterotrophic bacteria which rely on organic material in soil (including plant residues) for growth can be cultured on agar media. Selective agar is available for culture of different broadly-defined taxonomic groups, for example the fluorescent pseudomonads which are important rhizosphere bacteria. There are several methods available for examining the diversity of culturable bacteria including cellular proteins, fatty acids, and DNA fingerprints. Since the genetic material defines the identity of an organism, profiles based on DNA should be the most reliable method for identification, though not for indicating activity.

DNA fingerprints of individual isolates can be obtained using PCR with primers binding to different regions of the genome, enabling amplification of segments of different sizes which can be separated by gel electrophoresis. Identical or very closely related isolates generate identical band patterns. Only a small amount of DNA is needed for PCR: this can be extracted directly from colonies on agar, enabling large numbers to be screened. Fluorescent pseudomonad populations were investigated in the long-term Chemical Reference Plots at Rothamsted, comparing subplots which had received either no pesticides or a combination of five compounds over a 20 year period. PCR fingerprints of colonies isolated from the soil showed that the populations in the two plots were different but equally diverse. From 42 and 43 colonies from the pesticide-treated and control plots respectively, there were 16 and 19 different profiles (each representing one to seven isolates) with only six profiles in common to both plots[1]. It was difficult to compare profiles run on different gels and apparent that a better system for comparing and storing DNA fingerprint data was needed.

To achieve this, the fingerprinting method was adapted using fluorescently-labelled primers so that PCR reactions can be run on an automated DNA sequencer with a differently-coloured internal size marker in each track (Fig.39). This enables semi-automation of profile analysis so that large numbers of isolates can be compared. Fluorescent pseudomonad populations from the

Fig. 39 *PCR fingerprints made using flourescently-labelled primers with a differently-coloured internal size marker in each track.*



Woburn Market Garden Experiment were investigated from a sewage sludge-treated plot contaminated with heavy metals and a control plot treated with farmyard manure that was low in metals. Both were under grass. Fingerprints of 100 isolates from each plot showed only two in common with only a few occurring more than once in each plot. This contrasts with the findings on the pesticide-treated soil and appears to indicate a greater diversity in grassland soil at Woburn compared to soil under wheat at Rothamsted, but the significance, if any, is not yet clear.

(1)Nicholson, P.S. & Hirsch, P. R. (1998). *Journal of Applied Microbiology* **84**, 552.

Penny Hirsch - (IACR-Rothamsted)

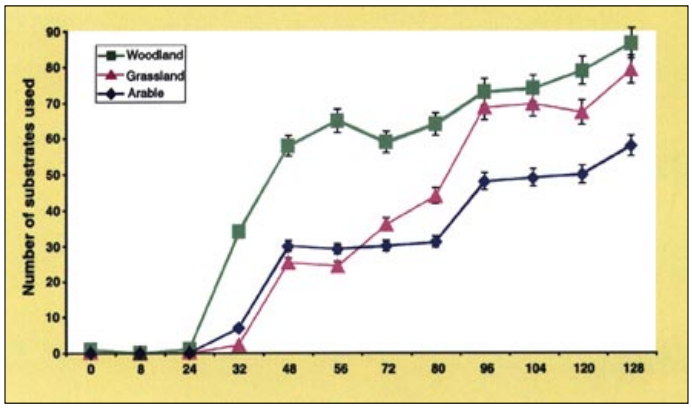


Fig. 40 *Changes in carbon utilisation profiles with land managment measured using BIOLOG™*

Functional diversity

Two approaches to observe and quantify the effect of changing land use on soil functional diversity have been compared: BIOLOG ™ (1) and Substrate Induced Respiration (SIR)(2). Both methods are expected to reflect the activity of the predominant heterotrophic fast-growing bacteria in the soil. BIOLOG ™ gives a metabolic fingerprint of the soil microbial community and reflects the capacity of microbes in soil suspensions to metabolise 95 different carbon substrates, as measured by the colour development of tetrazolium, a metabolic dye. SIR gives catabolic profiles showing the capacity of a soil to utilise a range of carbon and nitrogen compounds added to soil. An example of changing land use on BIOLOG ™ -derived functional diversity is given in Figure 40.

The Ley-arable experiment at Woburn Experimental Farm was used to test the application of these methods. Soils were sampled from a continuous arable treatment and from a soil that was ploughed for arable cultivation after an eight year ley. The soils were first sampled in October 1996, before cultivation, and again one year later, in October 1997, after both soils had grown winter wheat. The results from both BIOLOG™ and SIR were compared by Principle Components Analysis. Both techniques show similar trends in microbial functional diversity with the changing land use. There were clear differences in diversity between the continuous arable soil and that under the eight year ley when sampled before cultivation (Fig. 41). However, the signal from the grass ley had disappeared one year after cultivation. At this stage the characteristics of the two treatments were very similar as shown by either SIR or BIOLOG™. Interestingly, the Principal Components Analysis showed that the diversity characteristics of the continuous arable soil altered considerably during the course of one year.

(1)Garland, J.L. & Mills, A.L. (1991). *Applied and Environmental Microbiology* **57**, 2351.

(2)Degens, B.P. & Harris, J.A. (1997). *Soil Biology and Biochemistry* **29**, 1309.

Síle O'Flaherty - (IACR-Rothamsted)

Fig. 41 *The Woburn Ley-Arable Experiment*

Biochemical markers and chemical fingerprinting

Phospholipid fatty acids (PLFAs) and lipopolysaccharides (LPS) are signature biochemicals. Both are major constituents of the membranes of all living cells and can be used as biomarkers for soil microorganisms. We can identify fatty acids indicative of Gram positive and Gram negative bacteria, and eukaryotic biomass. It seems possible that the proportion of specific groups of fatty acids could be used to indicate when a population is under stress (Fig. 42). Various groups have been compared in soils that are either contaminated with heavy metals from sewage sludge or have lower metal concentrations following farmyard manure applications. The sludge-treated soils contained a larger percentage of hydroxy-substituted fatty acids, *trans*-isomer fatty acids and *cyclopropyl* fatty acids, and

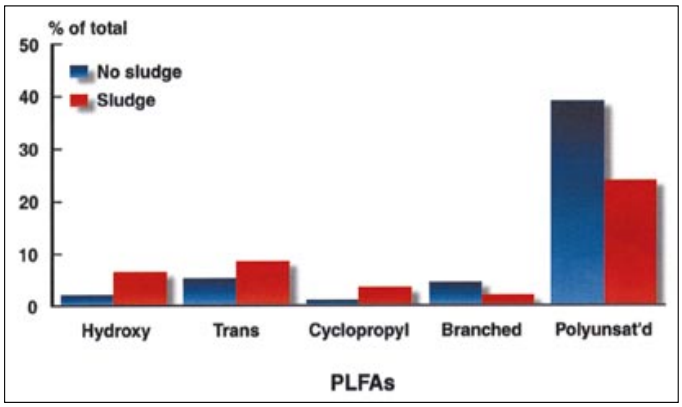


Fig. 42 *The relative proportions of selected phospholipid fatty acids (PLFAs) in soil with and without sludge, showing the effects of stress from toxic metals in the sludge*

smaller percentages of branched unsaturated and polyunsaturated fatty acids. We are continuing with this approach to see whether any of these differences can be used as an indicator of stress caused by metal pollution and whether or not other stresses have similar effects. We are also studying the usefulness of the ratio of the prokaryotic storage polymer, poly-β-hydxoxy butyrate to total PLFAs as a soil quality index.

Daniel Abaye - (IACR-Rothamsted)

Biodiversity and stress

It is often intuitively assumed that an ecosystem should contain as much diversity as possible and that any stress is likely to decrease biodiversity and thus ecosystem function and quality. Thus, in Figure 43 [1], Model A represents a simplistic relationship between biodiversity and stress. However, our research has shown that microbial communities sometimes respond as shown in Model B: i.e. biodiversity initially increases with stress. If B is the true model then taking two samples of soil at different degrees of stress could give a misleading picture: samples taken at 1 and 2 in Figure 43 would suggest an increase in biodiversity with stress; samples taken at 3 and 4 would suggest no impact of stress. Therefore, measuring only gross diversity, for example the number of genotypes, may be misleading as the community composition is very different at the different levels of stress. This may have implications for resilience of those communities and for soil functioning when further, or other, stresses are experienced.

[1]Giller, K. et al. (1998). *Soil Biology and Biochemistry* **30**, 1389.

Steve McGrath - (IACR-Rothamsted)

Indices of nitrogen loss

Values of individual parameters are often difficult to interpret in the context of soil quality. There may be no control or agreed value for a soil of good quality. One answer is to use ratios. The ratio gross N nitrification:gross immobilisation is a measure or index of potential N loss to the environment, often termed N saturation. It is measured using the ^{15}N pool dilution techniques described in last year's Annual Report. Figure 44 shows examples of this measured ratio for different land uses on a mineral and a peat soil. The indication in Figure 44 of N saturation and risk of loss under reseeded grass and arable peat soil is understandable in that cultivation causes the rapid mineralisation of much N, almost always too much for plants to utilise within a short period. However, N saturation in woodland, especially that on peat, suggests that there are other sources of N, particularly that deposited from the atmosphere. The woodland on mineral soil is Broadbalk Wilderness, where aerial inputs are known to be approximately 100 kg N ha^{-1} yr^{-1}. Because of this large input and relatively small uptake by the mature trees, the soil emits as much nitrous oxide to the atmosphere as the arable plot on Broadbalk receiving 288 kg N fertiliser ha^{-1} yr^{-1} [1].

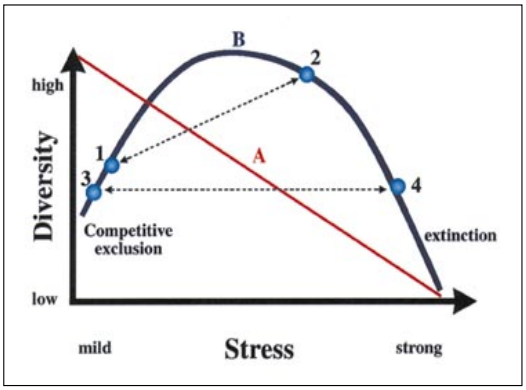
[1]Goulding, K.W.T. et al. (1998) *New Phytologist* **139**, 49.

Daniel Murphy - (IACR-Rothamsted)

Bioavailability

We previously established that potentially toxic metals introduced into soil in sewage sludge impair the activity of certain microorganisms, especially rhizobia. Presently, limits for each metal are based on the total content in soil. However, with different soils, even at similar pH values, there is often no relationship between the total concentration and toxicity. We are establishing limits on a more fundamental basis:- the bioavailability of a metal in the soil solution. For

Fig. 43 *A shematic model of diversity vs stress showing the two very different responses of diversity to stress and the problems of data interpretation*



example, it was found that the bioluminescence response (light output) of cells of a Pseudomonas biosensor declined as the toxic cationic form of zinc (free Zn^{2+}) increased in soil solution, and that two soils fitted the same relationship with soil solution metal concentrations (Fig. 8). The point of 50% inhibition derived from this curve was at 6 mg free Zn^{2+} l^{-1}, and we are investigating whether this holds for all soils. In contrast, the response to total Zn concentrations in the bulk soil showed distinct curves for each soil, further highlighting the appropriateness of free Zn^{2+} as a toxicity indicator.

Steve McGrath - (IACR-Rothamsted)

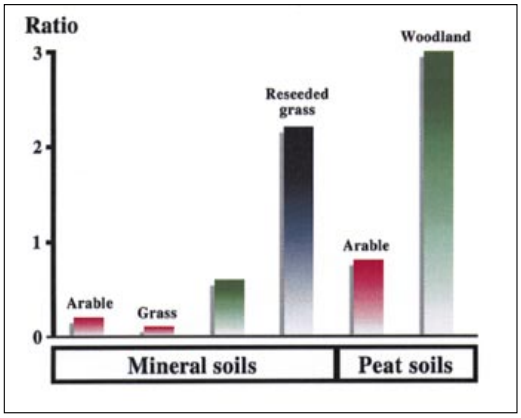


Fig. 44 *The ratio 'Gross nitrification:Gross immoblisation' as an indicator of nitrogen saturation and risk of loss to the environment. A ratio of 1 or more indicates saturation*