TOWARDS THE YEAR 2000 : DIRECTIONS FOR FUTURE NITROGEN RESEARCH

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ABSTRACT

Advances in knowledge concerning N in agroecosystems require a clear definition of achievable objectives and usable techniques. The impacts of computerization, molecular genetics and an increasing contribution by ecologists will play a major role in such advances. Broad goals could include complete utilization by plants of all mineral N and elimination of N losses to the environment. This will require plants with increased competitive capacity grown under management techniques that synchronize microbial release and plant uptake of soil N. I predict that the plant host, rather than the Rhizobium, will probably be found to be the limiting factor in many leguminous associations. This will require a greater research emphasis on the host. Techniques must be found for using legumes both as N sources and as weed competitors in alternative input agriculture ecosystems. Molecular genetics will affect N research in a number of ways. More basic information regarding plant-microbial interactions, microbial competition and nutrient cycling must be obtained before genetically engineered organisms can be released into the environment. It also will provide useful research tools such as genetic probes for specific microbial DNA in soil. Interdisciplinary projects will be required to ensure productive applications to field problems.

GOALS AND OBJECTIVES OF FUTURE RESEARCH

Advances in a field as important and at the same time as complex as that of N in agricultural ecosystems will depend on the recognition of research imperatives that can be successfully investigated, on the availability of useful concepts and on the applicability of imaginative techniques. The broad goals can be defined to include: (1) no losses of or pollution by mineral N, (2) increased plant competitiveness for N, (3) increased symbiotic fixation,

(4) synchronized release and plant uptake of soil or fertilizer N, (5) alteration of site characteristics in agroforestry, (6) companion or alternative N sources for high value crops that are grown in agricultural systems with lower utilization of pesticides.

Improved technology such as the measurement of the size of the microbial biomass (Smith and Paul 1987), automated rapid instrumentation for ^{15}N analysis (Marshall and Whiteway 1985) and the rapid identification of specific microorganisms in soil (Jansson _et al_. 1987) will have a major impact. Other advances have been made in measurement of gaseous N losses, the ecology and physiology of plant microbial interactions and the estimation of soil active N (Paul _et al_. 1985). Also, it is generally considered that advances in molecular genetics will be most readily applied to the plant-soil system through the activity of altered microorganisms (Halvorson _et al_. 1985). Soil microbiology and the study of N transformations therefore are now in a state of anticipation and excitement akin to that of a hundred years ago when the basic principles concerning N in agricultural ecosystems were being established.

It has been stated that the lack of basic understanding of the biological processes involved will be a limiting factor on future breakthroughs (Board on Agriculture 1985). It also is argued that the return from basic research will be greater than that from applied. Estimation of the annual rates of return to basic and applied research is very important but somewhat difficult to achieve. Using regression analysis that compared the ratio of an index of total output produced relative to inputs, (Evenson 1978) showed that in the 1927-1950 period basic science oriented research gave a higher rate of annual return [110%] relative to applied technology oriented research [95%] (Table 1). The opposite was true in 1948-1971 when technically oriented agricultural research gave a 2-3 fold greater return [106%] as compared to basic science oriented research [45%]. Fox's similar analyses in 1986 showed that crop research in the 1944-1983 period was equally affected by technical commodity-specific research and disciplinary biological research with a 180% return in both cases. It is of interest that during these years returns from animal science research were lower than those of plant sciences in both the basic and applied areas.

The justification for both applied and basic research in the plant sciences is very strong and consideration of the specific thrust of the research is probably more important than whether it is basic or applied.

METHODOLOGY IN NITROGEN RESEARCH

Multi-sample digestion and diffusion techniques have greatly increased the speed of handling samples for both carbon and nitrogen (Turner and Bergersen 1980). This, combined with automated methodology for conversion of ^{15}N NH_4^+ to N_2, has lead to reasonably priced ^{15}N analysis. The recent combination of an automated Dumas combustion furnace with gas chromatography and computer controlled mass spectrometer has made possible the direct analysis of both total N and ^{15}N contents of plant materials or soil samples (Marshall and Whiteway 1985). Elimination of Kjeldahl digestion, distillation or diffusion, concentration, and conversion to N_2, results in total N and ^{15}N analyses on a solid or liquid sample in five minutes with the capability of running 50 samples without operator attendance. The memory effects resulting from the retention of $^{15}N_2$ in the columns, the need for fine grinding and the six decimal place weighing of plant samples presents some difficulties; however, the ability to run a hundred samples in a working day on an instrument that provides ^{15}N analysis with a coefficient of variation (c.v.) of 0.2% and total N analysis with a c.v. of 1-2% will provide a major stimulus for N research (Table 2).

Table 1: Estimated annual rates of return to basic and applied research in U.S. agriculture.

Years	Type of research	Evenson (1978)	Fox (1986)
1858-1930	All agriculture	65	
1927-1950	Technical agricultural research	95	
	Science oriented research	110	
1948-1971	Technical agricultural research	106	
	Science oriented research	45	
1944-1983	Technical crop research		180
	Science oriented research		180
	Livestock research		130

Table 2: Automated simultaneous analysis of solid or liquid samples for total nitrogen and ^{15}N (or total carbon and ^{13}C).

Sample requirements	* 10-100 μg N * solid samples ground to <250 mesh * liquid samples sorbed on matrix such as Chromosorb
Throughput	* cycle time 300-400 sec, 100+ samples/day
Reproducibility	* ^{15}N, c.v.<0.2% (±0.001 at natural abundance) * total N, c.v. 1-2%
Unsolved problems	* typically 0.5-1% memory between samples * variation in isotope ratio with sample size - "Pressure effect"

MANAGEMENT OF NITROGEN CYCLING

Soil organic matter research is central to the understanding of the mineralization-immobilization reactions involved in N availability. We now realize that even in most N_2 fixing systems a significant portion of the plant's N comes from soil organic matter. On a global basis, the size of the mineralization-immobilization process is estimated as being 30 times that of symbiotic N_2 fixation (Table 3). Measurement techniques to further delineate microbial biomass and active N pools and to measure their dynamics therefore must continue to be zealously pursued.

Table 3: Global terrestrial N fluxes on an annual basis (Paul et al. 1985).

Source	N flux
	$g \times 10^{12}$
Soil N content	105,000
Plant uptake	$1{,}400^1$
Soil N mineralized	$3{,}500^2$
Symbiotic N_2 fixation	120
Associative and free-living fixation	50
Fertilizer N applied	65
Fertilizer N utilized	26
Combustion atmospheric inputs	22
Denitrification	135
Erosion-Leaching	85

1. 2% of 70 x 10^{15} g C photosynthesized; 2. 40% efficiency of uptake of mineralized N.

The ability to measure the flux of N through the microbial biomass and the soil active N fraction should lead to techniques for the management of the significant stores of N within these pools. Direct measurement of the C and N after lysing the cells holds promise but must be carefully calibrated. Incubation after fumigation releases more biomass constitutents but also requires careful calibration and more careful interpretation regarding the use of controls. An internal standard can be used to measure the effect of the fumigation-incubation on the release of nonbiomass C. Also it has been found that the ratio of CO_2-C produced after fumigation (C_f) relative to the CO_2-C evolved from a nonfumigated control (C_c) can be correlated to the results obtained with internal standards and direct counts (Horton et al. 1987). This allows the recalculation of a corrected control for published data and allows the application of a correction where ^{14}C internal standards are not available. Estimates of microbial N do not have as large a problem with the background controls as do those of microbial C; however, these estimates are very sensitive to the immobilization of mineral N during the incubation subsequent to fumigation. Voroney and Paul (1984) have shown that the flush of C relative to the flush of N (C_f to N_f) can be used to correct for this immobilization. This equation however also must be corrected for the effects of fumigation on the flush of carbon C_f (Horton et al. 1987).

The uptake of N by a large biomass and its conversion to a slowly available soil organic matter fraction explains: (1) very poor competitive uptake of N by plants such as forest trees and some grasses, (2) why nitrification inhibitors which keep the N in ammonium form, preferentially absorbed by microorganisms, have not been successful, and (3) the overestimation of soil N uptake in tracer experiments. The management of a microbial biomass such that it incorporates N at times of excess and releases it at times of maximum plant growth has merit from a theoretical basis but is not easy to accomplish under practical conditions. The tillage of agricultural soils, the incorporation of green manures at appropriate times, and management of forest soils by fire, are examples of successful management techniques. In addition, selection of plants with greater competitive capacity for NH_4^+ should have merit. The mycorrhiza and soil fauna also may play a role under such conditions.

The stress on the microbial partner in a large amount of the present N_2 fixation research is providing an excellent background of knowledge on microbial biochemistry and genetics and on plant-microbial interactions (Hodgson and Stacey 1987). It should also provide the necessary basis for breakthroughs in the molecular genetics of soil microorganisms. I may be proven wrong but I predict that, in most agricultural systems, plant controls on N_2 fixation will be the major limiting factor in increasing N_2 fixation. A greater stress on the plant will probably be necessary in future research imperatives.

Molecular biological techniques are aiding the study of recognition signals in plant microbial interactions which are mutually controlled. Aspects of symbiotic fixation such as competition between rhizobia within soil, and expression of the Nif genes are dependent on microbial activity. Areas such as root colonization, O_2 availability to the N_2 fixing system, and N uptake mechanisms are probably mutually controlled. The effects of C availability and allocation, stress avoidance (drought, frost, etc.), the effects of combined N and plant competitiveness in N nutrition reside more with the host than with the bacterium.

The effects of mycorrhiza and Rhizobia on plant morphology and photosynthetic efficiency are just starting to be understood with mycorrhiza appearing to have more effects than do Rhizobia (Harris et al. 1985). Mycorrhiza have a major effect through their role of supplying P and possibly via increased competitive uptake systems for soil NH_4^+ in N deficient systems. Mycorrhiza, however, can also act as plant parasites where symbiotic systems are not well established. The role of the associations must be investigated with greater imagination than has been possible in the past.

The diversity available in legume germplasm has been exploited only to a limited extent. Commercial agriculture is based on only a few species and interesting plants such as Sesbania which nodulate on both the stem and root and have N_2 fixing systems with higher O_2 and NO_3^- tolerance have been described but have not received the attention they deserve.

Future advances in N_2 fixation research will therefore be as dependent upon plant physiological studies and plant breeding as on the activities of the bacterial geneticist and agronomist.

Intensive crop management techniques will also play a major role in future research. Successful examples from tropical countries show development of double and triple cropping where only one crop used to be grown. In temperate climates, funds are now being allocated to develop lower input, more cost-effective cropping systems. One successful example involves seeding barley into a clover residue. This was interseeded with soybeans before harvest of the barley. The soybeans were then interseeded with winter wheat. The continuous cover eliminated most weed problems and chemical N input requirements were at a minimum, as was soil erosion.

MOLECULAR BIOLOGY AND NITROGEN CYCLING

The effects of molecular genetics on N cycling will in many cases be indirect. An example of this indirect effect will probably be found in the increased infusion of funds for basic research in soil microbiology and plant microbial interactions. Conversely, if soil microbiology and N cycling work does not show the significance of its contribution and the willingness to adapt, there could be a reallocation of manpower and resources to molecular biology. Table 4 lists some of the possible applications of genetic engineering to N cycling. The list is by no means exhaustive nor is it meant to indicate that practical applications are possible in these areas by the year 2000.

The application of molecular biology will have to take into account the already great genetic diversity and high microbial competition in soils; however, possibilities such as enhanced cellulose decomposition by associative N_2 fixers should not be overlooked. The introduction of a pathogen or pathogens that eliminate nitrifying organisms is probably technically possible but would no doubt have to be preceded by lengthy discussions that would involve social as well as scientific and agronomic considerations.

Some areas require new approaches. As previously mentioned, we may require more stress on the plant both as a controller of microbial activity and as a means of adjusting our management systems. Nitrogen fixation capacity in non-leguminous plants is often stated as an objective of molecular genetics. It may however be easier to leave the legume-rhizobium association intact and adapt the legumes to a wider array of crop characteristics. Can we produce a legume that has the yield potential, nutritional characteristics and climatic adaptation of maize while still maintaining high N_2 fixing rates? This would mean the production of a legume with corn kernels rather than the more often discussed production of N_2-fixing corn.

Table 4: The application of molecular genetics to advances in N cycling in agro-ecosystems.

A. Contribution to basic knowledge
   1) Genetic probes for specific microbial DNA in soils
   2) Concepts in microbial competition and plant-microbial interactions
   3) Application of more ecological theory to soil microbiology and nutrient cycling
   4) Enhanced knowledge of soil microorganisms such as Rhizobium genetics and physiology
   5) Genetic exchange among microorganisms

B. Microbial catalyses
   1) Sewage sludge degradation
   2) Industrial fermentation of natural residues
   3) Cellulose-lignin decomposing organisms such as associative N_2 fixing microorganisms

C. N_2 fixation
   1) Inoculation and infection
   2) Increased fixation capacity
   3) Fixation in presence of soil N
   4) Resistance to abiotic stress: salinity, drought, acidity, aluminium toxicity
   5) Rhizobium in non-legumes
   6) Adaptation of Frankia to crop plants
   7) Improved Azolla-Anaebaena associations

D. Improved nitrogen nutrition
   1) Inhibition or removal of nitrifiers
   2) Controls on mineralization-immobilization
   3) Controls on denitrification

E. Plant effects
   1) N_2 fixation in leaves
   2) Increased photosynthesis and C allocation
   3) Altered rooting characteristics
   4) Increased plant competitiveness for N
   5) Altered plant constituents affecting decomposition and allelopathy
   6) Incorporation of greater yield and altered starch contents in N_2 fixing legumes

ACHIEVING OUR GOALS

The complexity of plant microbial interactions and soil organic matter transformations will continue to hinder activity and provide excuses for a lack of major breakthroughs. One must question whether our present scientific system is capable of rising to the challenge of effectively moving N agro-ecosystem research into the 21st century. We have access to an extensive number of journals that accept both original publications and reviews. Do we have too many journals? One usually has to read two to five associated articles to determine what is happening in a particular laboratory. Some of this comes from our need to slowly adopt techniques while we apply them to the practical questions. The methodology for biomass measurements is a case in point. It took years of study in a number of laboratories to develop methodologies applicable to most soils. In the meantime, a large number of papers have been published utilizing incomplete methodology. Fortunately corrections to some of the original work can be made. However, some of the studies will probably have to be repeated as we try to get more meaningful numbers rather than comparative data.

A number of good review articles and books on soil organic matter are now available (Stevenson 1982; Aiken et al. 1985) and the re-entry of chemists into the field is producing a re-evaluation of much of the data. However I believe that not as many new concepts as are necessary to develop this important field are now appearing. We tend to accept publication of localized data. This can be argued as being necessary because of our soil type and climatic variability. One must however question whether this allows the investigators to rest on their laurels and not strive as much as they should for new concepts that create truth and knowledge.

Our present scientific establishment requires us to spend a great deal of time chasing a limited resource base and then to publish localized results rather than striving to improve the larger picture or to develop the basic concepts that are required for future breakthroughs in our science.

The need for more rapid advances in our field may require the establishment of fairly large research centers that concentrate interdisciplinary studies applicable to more than one site. Such centers would include studies of both the basic and applied sciences. Molecular biology together with automated instrumentation should supply a large number of the needed research tools. These tools together with the expanded application of ecological principles and mathematical modelling applied to systems agriculture should provide the background for the research necessary to achieve the objectives set out in the earlier part of this paper. If in the next 13 years we can set the stage for such reorganization and recognition of our science, we can ask the younger people entering the 21st century to rise to the challenge of increasing the efficiency and utilization of nutrients while decreasing the pollution potential of our environment.

A new factor that has entered our scientific scene to an extent that is greater than that previously encountered is the role of society. Legislation such as that proposed in Iowa where all fertilizer and pesticide sales would be taxed to pay for activities in pollution abatement and research into lower input agriculture will, if enacted, have a profound effect on N research in that State. This political activity is not surprising if one considers that it is claimed that one third of Iowa wells now have NO_3-N levels greater than those set for toxicity of NO_3^--N.

The role of public opinion and ethics in the release of genetically engineered organisms is also greatly affecting our research and management potential in this field. Our science, while continuing to be exciting and well supported, will have to take into consideration many more economic, environmental and political impacts than we have in the past.

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