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ABSTRACT

Use of labeled ^{15}N techniques can help improve and refine knowledge required to understand the dynamics of the N cycle under agricultural systems. The examples reported in this paper illustrate that, with creativity and careful planning, use of ^{15}N-enriched or depleted fertilizers can be used in many types of N experiments with various crops, cropping systems, and in many types of agroecosystems. The objective is to report examples of a wide range of field studies that researchers can hopefully draw upon to design and utilize N isotope techniques that accomplish the objectives for their own research. Examples of microplot studies with and without physical barriers are discussed along with some of the strengths and limitations of each approach. Even though ^{15}N technology offers considerable opportunity to understand N cycling, it too has limitations to its use. Types of fertilizer ^{15}N materials and methods for applying ^{15}N materials are discussed including some discussion about the use of ammonium versus nitrate forms; literature sources are provided for additional reading about the use of ^{15}N techniques.

INTRODUCTION

Nitrogen (N) is perhaps the most important nutrient element used in agriculture. Inputs of N fertilizers are essential to growing of crops and feeding of the World's populations. Fertilizer N use was essential to the set of technologies that dramatically increased food production in developing countries during the "Green Revolution" (1960 to 1980). For the World to maintain adequate food supplies requires that crops be supplied with N in the proper amounts and at the proper times to carry out their photosynthetic and metabolic processes. Application of excess N increases the potential for its loss into the environment by leaching, denitrification, and volatilization. Such losses often have the potential to cause severe effects to the environment. An important strategy to decrease the potential negative impacts of fertilizer N on the environment is to improve the efficiency of its use.

Nitrogen tracer techniques allow quantitative measurements of: (i) differences in fertilizer N use efficiency (FNUE) between sources, placement, and time of application, (ii) N-transformation processes, (iii) uptake of indigenous soil-derived and residual mineral N sources versus the ^{15}N labeled fertilizer, (iv) N recycled from the residues of a preceding crop, and (v) the fate and transport of ^{15}N-labeled fertilizer. The term "^{15}N-labeled fertilizer," will be used to include either ^{15}N-enriched or ^{15}N-depleted fertilizers since both can be used in tracer experiments. Field experiments using ^{15}N-labeled fertilizer N vary from those using only single plants, through small one row plots, lysimeters, and microplots. Societal concerns about the impacts of N fertilizer practices on water, air, and environmental quality are increasing and there is a strong need to develop technologies whereby FNUE is improved. Techniques for the experimental use of ^{15}N-labeled fertilizer under field conditions can contribute to the development of such improved technologies. However, it is necessary to be able to conduct studies using ^{15}N-labeled fertilizer under conditions that can realistically represent management practices that are used by producers. Use of such research techniques helps improve soil and crop management concepts under different ecosystems, result in improved FNUE, and decrease environmental problems associated with N-fertilizer management. The objective of this paper is to provide selected examples where innovative techniques for ^{15}N-labeled fertilizers have been used in field studies and experiments designed to evaluate FNUE and understand soil N dynamics.

FERTILIZER ^{15}N MATERIALS

Highly enriched ^{15}N materials are expensive. Their use in field plot experiment is most appropriate where only small amounts of ^{15}N are applied. These very small amounts generally do not result in a response from the system being studied, i.e., a plant-yield increase or other notable effect. Lower ^{15}N enrichment in fertilizer materials are less costly per unit of total N, but are still expensive. The use of ^{15}N-depleted fertilizer material is the least expensive alternative. However, lack of isotope enrichment in studies that use ^{15}N-depleted materials may limit or prevent the detailed examination of N transformations, such as immobilization and mineralization. Commercially (Isotec, Inc. in Miamisburg, OH),[1] it is possible to obtain ^{15}N labeled or depleted material in ammonia (NH_3), ammonium (NH_4^+), nitrate (NO_3^-), and urea [$CO(NH_2)_2$] forms. The NH_3 can be obtained in gaseous form and the NH_4^+ and NO_3^- forms can be obtained as various salts, including as ammonium nitrate (NH_4NO_3) with either the NH_4, the NO_3, or both carrying the ^{15}N or ^{14}N label. The ^{15}N enrichment levels commonly available are 5, 10, 60 and about 99 atom percent (%), while ^{15}N depleted materials are usually available as 99.9+ atom % ^{14}N (<0.1 atom % ^{15}N). By comparison the of atmospheric dinitrogen (N_2) is about 0.366 atom % ^{15}N (Porter and Mosier, 1992).

Depleted ^{15}N

The potential to design experiments using depleted ^{15}N are illustrated in studies by Broadbent (1980), Patrick et al. (1984), Porter (1995), Russelle et al. (1981), and others. An example where depleted ^{15}N was used is that by Porter (1995), where he evaluated N-fertilizer carryover, leaching, and uptake under continuous irrigated corn (*Zea mays* L.) on a Weld silty clay loam (fine, montmorillonitic, mesic Aridic Paleustoll) near Akron, Colorado. Depleted ^{15}N fertilizer was applied annually for three yrs at four rates to continuous corn, including two N rates that were excessive, under three irrigation rate regimes, one of which was excessive. Beneath the undisturbed soil profile of each plot, at a depth of 1.22 m, a "vacuum trough" extractor was installed to measure weekly percolate and NO_3^- concentration derived from fertilizer in the percolate. The study showed most of the measured fertilizer-derived NO_3^- in the percolate occurred the third year under the highest N-fertilizer and water application rates and that fertilizer-N rates required for near-optimum plant yield minimize the accumulation of residual NO_3^-.

^{15}N Labeled Slow-Release Fertilizer

Use of nitrification inhibitors and/or slow release fertilizers can increase FNUE, especially where the potential for losses of N by leaching or denitrification are high. As reported by Hauck et al. (1994), only a few studies have been made

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[1] Trade and company names are included for the benefit of the reader and do not imply endorsement or preferential treatment of the product or the manufacturer by the authors or the USDA.

with these types of ^{15}N-labeled materials, e.g., with urea forms (Brown and Volk 1966), oxamide (Westerman et al. 1972), and oxamide and isobutylidene diurea (Rubio and Hauck 1986). A recent field study using nitrification inhibitors and slow-release fertilizers is that by Delgado and Mosier (1996). They determined the FNUE and nitrous oxide (N_2O) and methane (CH_4) fluxes that occurred with irrigated spring barley (*Hordeum vulgare* L.) when urea, urea plus the nitrification inhibitor dicyandiamide (U + DCD), and polyolefin coated urea (POCU) were used. Within each of the above treatments microplots were established that received 90 kg N ha^{-1} as ^{15}N-enriched urea or POCU. The recovery at harvest of ^{15}N fertilizer in the plant-soil system was 98, 90, and 85% and yields were 2.2, 2.5, and 2.7 Mg ha^{-1} from POCU, urea, and U + DCD, respectively. Potential was shown by both the nitrification inhibitor and the controlled release fertilizer. However, more work in needed under different crops, soils, and with improved formulations of the POCU to better match crop growth demands for N.

USE OF ^{15}N-LABELED FERTILIZER FOR FIELD RESEARCH

Two overall approaches for using ^{15}N-labeled fertilizer in field experiments will be addressed. The goal of both approaches is to mimic conditions and often the practices that producers use. Another goal is to understand the N dynamics in the soil-plant system and the factors that affect the retention, loss, and plant uptake of N. These two general types of experiments are those that place physical barriers around the microplot within which the ^{15}N-labeling experiment is conducted and those that do not. Both types require the use of ^{15}N-labeled materials and access to the laboratory equipment required for isotopic analysis. Certain protocols need to be followed to reduce potential cross contamination problems and to allow the collection of high quality data for interpretation and analysis as described by Porter and Mosier (1992) and Hauck et al. (1994). Basic problems encountered with the use of ^{15}N-labeled fertilizers in field studies are further identified by Hauck et al. (1994), to include (i) nonuniform distributions of N within the field, such as from the differences in soil horizons and vertical distribution, and (ii) natural and experimentally induced variations in the composition of subsamples.

Application of ^{15}N-Labeled Fertilizer in Field Studies

^{15}N-labeled fertilizers can be applied as broadcast, as a spray, by mixing with, and by injecting into the soil. The user of the ^{15}N-labeled fertilizer must be very aware of the form of the ^{15}N fertilizer and of potential loss mechanisms including, volatilization, leaching, and denitrification which can cause significant balance sheet errors unless they are measured. When NH_4^+ salts or urea are applied on a soil surface it can be important to immediately till the soil several inches deep to help reduce NH_3 volatilization, especially if applied to calcareous soils, as discussed by Porter and Mosier (1992). A uniform application is difficult to achieve when applying fertilizer salts by hand. Removing the soil and mixing it with the fertilizer is probably the best way to achieve a high degree of uniformity. Uniform mixing of ^{15}N-labeled fertilizer is possible when microplot size is limited to small cylinders, but the labor associated with mixing is increasingly costly as microplot size becomes larger. Where soil surface disturbance is undesirable, such as in permanent grass (rangeland, pasture or meadow), small cylinders offer the advantage of having a small surface area, being confined, and allowing the ^{15}N-labeled fertilizer to be applied in dissolved form or broadcast and then water applied to simulate the effect of precipitation or even irrigation. Methods for applying ^{15}N-isotopes into soil and plants are discussed by Porter and Mosier (1992). Hauck, et al. (1994) provided an additional strong review of the consideration required for the overall conduct of ^{15}N-labeled fertilizer research including the preparation of the materials, calculation of ^{15}N-labeled fertilizer needs, and the collection and preparation of samples.

Anhydrous Ammonia

Anhydrous NH_3 is widely used in cropping systems. However, the use of ^{15}N-labeled anhydrous NH_3 is generally avoided by researchers because conventional NH_3 applicators are difficult to calibrate for uniform rates of application on plots as small as microplots. Accurate application methods at point locations in soil have been proposed by Papendick and Parr (1965) and by Bremner et al. (1981), but the application at point locations often do not achieve the type of distribution found when NH_3 is applied in bands by conventional methods. Sanchez and Blackmer (1987) recognized the need to apply NH_3 to microplots and described a method that permits precise application of anhydrous NH_3 in bands to microplots. Their method involves placing a stainless-steel capillary tube in the soil where the NH_3 is to be banded, attaching this tube to a cylinder of NH_3 and then pulling this tube through the soil with deposition of NH_3 as an even band. In addition the procedure has the advantage of allowing the method to be used with mixtures of ^{15}N-labeled anhydrous NH_3 and nitrification inhibitors. With this method the soil environment at the point of application is generally representative of conditions that occur with conventional application of anhydrous NH_3.

Buried Drip Tubing

Buried drip irrigation allows growers to maximize both N- and irrigation water-use efficiency while reducing the potential for ground water contamination. This is accomplished by the precise delivery of N and water directly to the plant root system. An apparatus to apply ^{15}N-labeled fertilizer is described by McGee et al. (1995). The apparatus is constructed with 1.3 cm polyvinyl chloride (PVC) pipe and fittings and 1.3 cm brass ball valves and can be installed directly into drip tubing systems. McGee et al. (1995) evaluated the apparatus with leaf lettuce (*Lactuca sativa* L.) planted in 4.1 by 53.3 m beds. Microplots (2 by 1.02 m) within the beds were established by placing the fertilizer injection apparatus into the drip tubing prior to the first N application. Two N-fertilizer rates (71 and 176 kg ha^{-1}) were replicated three times. These low- and high-N rate plots received 1.60 and 1.15 atom % ^{15}N urea solution, respectively, applied at four times during the growing season. The fraction of total uptake from the ^{15}N-labeled fertilizer was calculated using the method described by Sanchez et al. (1987). Uniform recovery of ^{15}N by plants within the subplots was observed and the method has excellent potential to be adapted and/or modified for additional studies.

Methodology Used by the Author

To overcome difficulties associated with ^{15}N-labeled fertilizer application, a technique was developed to allow highly uniform application rates, different rates of application, and to have the potential for use at distant research sites. Initial results of the application technique are described by Follett et al. (1991), and the method hereafter referred to as the "Follett et al. method." The method is adaptable for use with ^{15}N-labeled NO_3^-, -urea, or -NH_4^+ fertilizer materials. When urea and NH_4 materials are used, they should be incorporated soon after application to minimize volatile NH_3 loss.

To achieve the desired application rate requires knowledge of the microplot area so that the amount of ^{15}N-labeled material to dissolve in a known volume of water can be determined. The ^{15}N-fertilizer salt must be completely dissolved. Quantitative application is accomplished by pouring a predetermined amount of the ^{15}N solution into a tubulated polyethylene bottle that is reinforced with metal bands (such as worm-drive hose clamps). The bottle is connected to a compressed air cylinder through a screw cap closure, modified with a bulkhead tubing fitting (Fig. 1), to allow the bottle to be maintained at a constant pressure of 0.14- to 0.20 MPa (Fig. 2). Once pressurized the solution is sprayed onto the microplot area until the bottle is completely empty by using a hand held spray wand made of commercially available parts (Figs. 1 and 3). Adjustment of the amount of liquid to a proper volume allows the spraying of the ^{15}N solution across the microplots to be repeated (3- to 5 times) in different directions over the microplot to assure uniform application.

Inexpensive non-^{15}N labeled fertilizer material, or even water, can be used to calibrate the spray apparatus to an area equal to that of the microplot before using the ^{15}N enriched material on the actual microplots. This apparatus has been

Figure 1. Equipment used for applying ^{15}N labeled material to microplots that do not have barriers placed around them. Included from left to right are a bucket for carrying the gas cylinder, the spray wand used for spreading the ^{15}N labeled material, the polyethylene bottle for the liquid that is sprayed onto the microplots, hoses and the gas cylinder that supplies the pressure.

used successfully to apply ^{15}N enriched material onto grass plots (Fig. 3), high residue (Fig. 4) or low residue (Fig. 5) plots, and under high-wind conditions with wind speeds of up to 30 km hr^{-1}. The Follett et al. method has generally been used to broadcast apply fertilizer ^{15}N material on the surface, but by pre-making furrows with a hoe and then recovering with soil after application, can be used to band ^{15}N-labeled fertilizer. Transport of the equipment for hundreds of km by surface vehicle is feasible. If air travel is necessary, transport is only required for the air pressure gauge, polyethylene bottle, hoses and spray wand because compressed air can normally be rented at the destination.

The procedure is versatile in that it can be used for enrichments ranging from 0.0- to $<$5.0- to $>$10- to 98+ atom % excess. At lower enrichment, the ^{15}N is applied as a labeled fertilizer. Where 98+ atom % excess material is applied, it is generally for ^{15}N tracer purposes. Illustrations of the versatility of this apparatus and procedure are described in some of the below reports. In testing the procedure, Follett et al. (1991) determined that dry matter yields and plant-N uptake by win-

Figure 2. Gas cylinder with pressure regulator and hose for attachment to spray equipment.

ter wheat (*Triticum aestivum* L.) resulting from applications of $K^{15}NO_3$ dissolved in liquid and sprayed onto the soil surface inside of microplot boundaries were not statistically different from those resulting from the application of commercially available granular KNO_3 fertilizer applied around and outside of the boundary of the microplots. The methodology allows microplots to be incorporated into field plot experiments and, under careful management, to be used directly in producer fields.

^{15}N-LABELED FIELD-STUDIES USING PHYSICAL BARRIERS

Physical barriers delineate the boundary around small plots (microplots) by confining and isolating them from the surrounding soil. Thus, they eliminate the lateral movement of ^{15}N-labeled fertilizer in the soil and prevent it from being taken up by outside plants. Comparison of measurements made for microplots with physical barriers versus those without is rarely reported. However, Saffigna (1988) found similar ^{15}N recoveries with wheat in 0.5 m diameter cylinders when compared to 1 m^2 unconfined microplots. Use of physical barriers for ^{15}N field

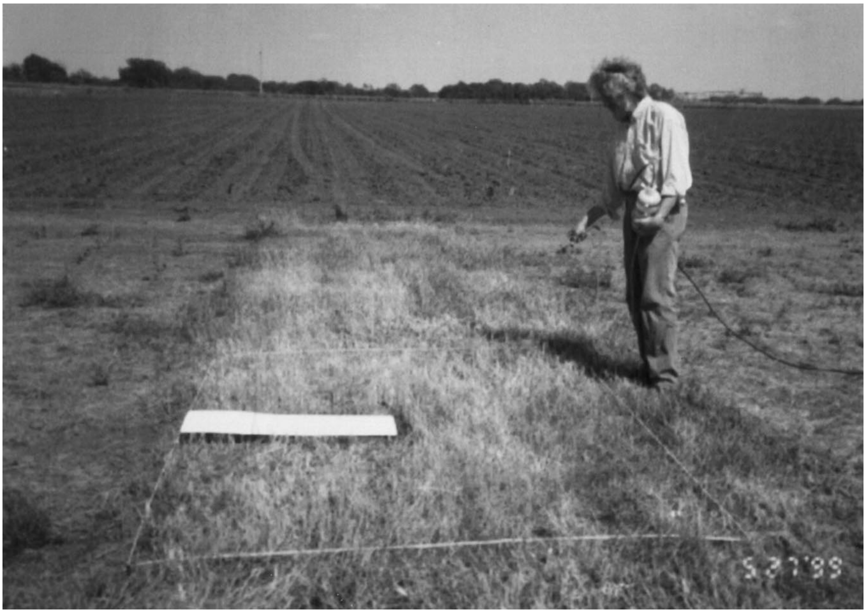

Figure 3. Application of ^{15}N labeled material to a bromegrass sod microplot. The rope on the ground outlines the microplot and the white board within the microplot shows one of the areas for later subsampling to be done during several seasons.

studies have many applications, especially for small stature crops such as small grains and for grasses as described by Legg and Meisinger (1982). The cost of ^{15}N-enriched fertilizer often results in the decision to use physical barriers because a smaller microplot size requires less ^{15}N-labeled material. Other considerations are the statistical design of the study, number and type of samples to be collected, crop (or plants) used, duration of the study, and the ecosystem within which the study is to be conducted. Physical barriers need to allow sufficient soil volume for the root systems of the growing plants to develop normally. If the plants in the study have larger root systems, the area confined by the physical barrier needs to be larger. Increasing the size of the area confined within physical barriers increases the difficulty of installing and removing them and to greater soil and root zone disturbance during installation and/or removal.

Sanchez et al. (1987) summarized a number of concerns about using physical barriers, including the following. Even though barriers eliminate problems associated with lateral movement, they themselves may introduce artifacts that affect fertilizer N recovery by plants. These artifacts may result from the inability of the root systems to achieve normal distribution and size, disruption of the mac-

Figure 4. Corn residues into which winter wheat has been no-till seeded and which has had ^{15}N labeled material applied for a long-term study in Mexico.

ropore system and/or the creation of artificial pores that may influence aeration or movement of water and solutes, and concentrated water infiltration down the cylinder walls, particularly in cracking clay soils. In addition, normal tillage and other cultural practices associated with farming are often incompatible or difficult to incorporate into the design of field studies that use microplots with physical barrier placed around them.

Sudangrass (*Sorghum sudanese* [Piper] Stapf)

An early study that was unique in helping establish some of the parameters that needed to be considered for an ^{15}N study that used physical barriers was that conducted by Carter, et al. (1967). They established that a single cylinder, approximately 30 cm in diameter and pressed into the soil 45 to 60 cm, could serve as a satisfactory microplot. The study was conducted on a sandy loam soil in Alabama under a humid soil moisture regime (Soil Survey Staff 1998) in which the test crop was sudangrass. Total ^{15}N fertilizer recovery after 8 weeks ranged from 88 to 96%, but dropped to as low as 77% after 10 months. Recovery was

Figure 5. Plowed bean field into which winter wheat has been planted. The soil is a Vertisol and the dark rectangle on the soil is a moist area where ^{15}N labeled fertilizer has just been applied, while outside of the moist area natural abundance N fertilizer has previously been applied. The natural abundance material was kept off of the area that the ^{15}N labeled material was later applied to by mean of a plastic cover.

greater with an $^{15}NH_4^+$ than with a $^{15}NO_3^-$ fertilizer source and better where there was a growing sudangrass crop than where the microplots were fallow.

Small Grains

Confined microplots are readily adaptable under field conditions for studying N budgets under small grains. Tomar and Soper (1981) used open-ended, 35-cm diameter iron cylinders that were pressed into the soil to a depth of 25 cm. They then grew a crop of barley (*Hordeum vulgare* L.) to study different methods of N and organic matter placement. Labeled urea (19.15 atom % ^{15}N) was either banded at 10-cm soil depth or surface broadcast at the rate of 100 kg N ha^{-1}. The study was conducted on a Hochfeld loamy sand, under Canadian soil classification an Orthic Black Chernozen (Udic Haploboroll). The organic matter source was oat straw (*Avena sativa* L.) at 5000 kg ha^{-1}, mixed into the soil to a depth of

10 cm or surface applied. Nitrogen uptake and barley yield were greater from the banded than from the broadcast urea and uptake for both methods was enhanced by adding the organic matter to the soil. Fertilizer N recovery in plant tops was between 22.8 to 42.8% and 42.0 to 53.1% for surface broadcast and banded treatments, respectively. Between 83 to 87% and 82 to 95% of the applied N was accounted for, in the soil plus the plant material, with the surface broadcast and banded treatments, respectively. Leaching was considered negligible and thus the unaccounted for differences of 13 to 15% were considered to have resulted from gaseous losses.

In a study by Craswell and Martin (1975), 16 cm diameter PVC pipe was cut into 67 cm lengths and placed in the soil to a depth of 60 cm. The soil was a Waco clay, a heavy Australian clay soil. The top 15 cm of soil was removed and homogenized and added back to the cylinders in three increments, each wetted to 56% moisture as they were returned to the cylinders. Ten atom % ^{15}N-labeled calcium nitrate was dissolved and applied as a liquid solution on top of the first (10 to 15 cm) increment of returned soil at 112 kg N ha^{-1}. Microplots were maintained fallow and after 16 weeks, during which 190 mm of rain fell, 97.7 ± 2.4% of the added ^{15}N was recovered. The above fallow study was compared by the authors to two other sub-studies reported in the same paper in which they grew winter wheat (*Triticum aestivum* L.) in cylinders prepared similarly to those used for their fallow study, but with the cylinders wrapped in bituminized paper and set out in the field. The results and comparisons made by the authors led them to conclude that near-total recoveries of ^{15}N in the field contrast with losses that had been reported for greenhouse and pot studies. Secondly, they identified the need to evaluate the potential of volatile losses of ^{15}N from the wheat plants themselves, whereas most workers up to that time had looked only to the soil for the mechanism of loss.

Native Grasses (Rangeland)

An early and perhaps classic study of the rates and the routes by which N, once taken up by the plant, moves into litter and soil compartments pools and from them to new plant growth was reported by Clark (1977). Using ^{15}N tracer, and physical barriers for a study conducted in Colorado in a semiarid soil moisture regime (Soil Survey Staff 1998), 64 individual cylinders (20 cm diameter and 40 cm long) were driven to a depth of 36 cm into otherwise undisturbed shortgrass prairie. The vegetation was dominantly blue grama (*Bouteloua gracillis* Lag.). To each cylinder 83.16 mg of N as KNO_3 (25.8 kg N ha^{-1}, and containing 66.53 mg of excess ^{15}N) was added in 10 ml of water followed immediately with an additional surface irrigation of 1.5 cm of water. Selected cylinders were removed over the next five growing seasons and the plant and soil material separated for analy-

Figure 6. The top of a metal cylinder that has been placed into the soil for use in a long-term grassland study of ^{15}N dynamics in Colorado.

ses. Among Clark's (1977) conclusions were that, in the year of application, ^{15}N was removed primarily in the green herbage and that there was late season translocation of N from aboveground to belowground plant parts. The pulse of ^{15}N peaked in the aboveground dead compartment in the winter months following the first and preceding the second growing season. He observed that the transfer of ^{15}N from aboveground litter occurred principally during the second growing season, the ^{15}N content of crowns and live roots did not change significantly during the five seasons, and that the ^{15}N content of senescent and detrital roots increased significantly during the same interval.

Delgado et al. (1996b) conducted a study in Colorado, under a semiarid soil moisture regime, on paired backslope (15% clay) and footslope (27% clay) positions on a catena vegetated dominantly by blue grama. Stainless-steel cylinders, 10 cm in diameter (Fig. 6) and 40 cm long, were driven 38 cm into the soil in 1981 and 1982 and then removed for sampling and analyses in 1992 (Fig. 7). After 10 yrs, ^{15}N recovery of the applied N decreased at both topographic positions to 85% in the footslope position and 27% in the backslope position. The earlier results of Clark (1977) and those by Delgado et al. (1996b) illustrate not only the strengths of the confined-microplot technique for long-term studies, but also the

Figure 7. Single cylinder that has been removed after 10 years from a long-term grassland study of ^{15}N dynamics in Colorado. The cylinder still contains soil that will be removed in layers for laboratory analyses.

power of using ^{15}N labeling to study % ^{15}N recovery, turnover rates of N in the soil organic matter, and the partitioning of N between the soil and plant material. Much was also elucidated by the use of ^{15}N about the long-term effect of soil texture, slope position, and grassland systems on the retention of N within this type of ecosystem.

Mountain Meadows

In a study using ^{15}N-labeled urea versus NH_4NO_3, Delgado et al. (1996a) applied 168 kg N ha^{-1} in the fall or spring onto confined microplots. The microplots were constructed of 20 cm diameter PVC pipes driven 20 cm into the soil of a high-altitude wet (mountain) meadow in Wyoming. The meadow species at the site included timothy (*Phleum pratense* L.) and bluegrass (*Poa pratensis* L.). Using "wild-flood" irrigation, typical of mountain-meadow management, water stood continuously over the plots and remained at a water-filled pore space of above 90% for periods up to four week during 1993, but for periods of less than one week during 1994. Removal of the cylinders at selected times allowed analyses of the total soil and plant materials. Nitrogen fertilization significantly increased the harvested forage biomass. A N budget for the cylinders showed total losses of ^{15}N-labeled fertilizer that averaged 58, 40, 43, and 29% for the fall-applied urea, spring-applied urea, fall-applied NH_4NO_3 and spring-applied NH_4NO_3, respectively.

Lysimeters

Another type of physical barrier within which ^{15}N-labeled fertilizer studies can be conducted are lysimeters. Cookson et al. (2000) reported the use of undisturbed-monolith lysimeters in New Zealand. The objective of the study was to measure leaching losses of N from unfertilized and fertilized lysimeters under a ryegrass crop that was sown the previous autumn. Before sowing to ryegrass, the vegetation on the lysimeters was killed and the soil hand cultivated to a depth of 15 cm. Three rows of perennial ryegrass (*Lolium perenne* L.) were sown in 15 cm rows across each monolith at the rate of 10 kg seed ha^{-1}. Labeled urea (10.5 atom % ^{15}N) was applied in autumn as at 0 and 50 kg N ha^{-1}. The addition of ^{15}N-labeled fertilizer did not significantly increase nitrate leaching losses above the check treatments. However, a trend whereby N fertilization tended to increase the soil-derived N contained in leachate coming from the bottoms of the lysimeters was reported. The soil-derived N contributed 78 and 88% of the nitrate leached beneath fertilized lysimeters in 1996 and 1997, respectively. Warmer weather and wetter soil conditions during 1997, compared to 1996, resulted in an increased release of soil-derived N in 1997.

^{15}N FIELD STUDIES THAT DO NOT USE PHYSICAL BARRIERS

Micro-plot Size

Problems associated with lateral movement of ^{15}N-labeled fertilizer are especially important in studies that do not use physical barriers. The high cost of the ^{15}N-labeled fertilizers encourages the use of the smallest possible plot size and size of the microplots is determined largely by the amount of lateral movement. Studies without physical barriers around the plots usually require that all roots of plants being measured are grown in soil in which the ^{15}N-labeled fertilizer distribution is the same as it would be if they were instead grown in a very large treated area. The root system of corn plants can typically have a lateral root growth of 50- to 80 cm from the crown (Allmaras and Nelson, 1971; Follett, et al. 1974). When physical barriers are eliminated lateral N movement and/or lateral root growth largely determine how small the microplot must be before significant error is introduced. Olson (1980), using ^{15}N-labeled fertilizer that was surface broadcast and then incorporated into a silt loam soil, concluded that ^{15}N uptake data by corn could be accurately obtained by sampling the center row of a three- row plot (71 cm rows) and leaving 71 cm of unsampled border at each end of the center row, but that for residual ^{15}N studies the microplot size should be increased. Similar observations and recommendations were later made by Jokela and Randall (1987) in Minnesota (76 cm rows with 38 cm of border at the ends), and Stumpe et al. (1989) in Nigeria (75 cm row spacing and 50 cm of border at the ends). Powlson, et al. (1986) evaluated wheat grown in 2 m by 2 m microplots (twelve 16.7 cm rows) in England advised sampling the center six rows and leaving a 50 cm border on each end. Follett et al. (1991) advised that the minimum microplot size was 1.5 m by 1.5 m with fall-applied ^{15}N-labeled fertilizer on winter wheat.

The lateral movement of the ^{15}N-label by wind or water, by translocation in plant tissues (and residues), and by gaseous N transport may also need to be considered under certain situations such as for experiments that remain in the field for long periods of time. ^{15}N-labeled fertilizer studies that do not use physical barriers are important because they can allow normal cultural practices to be used. However, the possibility is introduced that cultural practices themselves will increase or even be the most serious source of lateral movement of the ^{15}N-labeled fertilizer and therefore a larger plot size is required, increased amounts of ^{15}N-labeled fertilizer are needed, and higher costs are incurred to conduct the experiment. The following are examples of ^{15}N-labeled fertilizer field-studies conducted without the use of physical barriers.

Rainfed Corn

Sanchez et al. (1987) discussed the theory whereby lateral movement of N can be detected by performing isotope ratio analyses on the tissues of plants growing near a border between two adjacent plots. The adjacent plots were fertilized at a common rate, but one of the plots had ^{15}N-labeled fertilizer applied and the other had non-labeled fertilizer N applied. In theory, a plant positioned exactly on the border between the two plots should take up half of its N from the plot having ^{15}N-labeled fertilizer and half of its N from the plot having unlabeled N fertilizer. This study was used to develope the mathematical relationship of the symmetry about the border for plants located equi-distant inside and outside of the ^{15}N-labeled fertilizer plot. The study further evaluated distant relationships for plants growing either far enough inside of the ^{15}N-labeled fertilizer plot that all of N taken up is ^{15}N-labeled or far enough outside that all of the N taken up is non-^{15}N-labeled. The authors were able to assess lateral movement in a direction parallel to the corn rows and identified that even in the absence of lateral movement of fertilizer N by mass flow or diffusion in the soil, plants growing in proximity to the ^{15}N-labeled fertilizer plot will take up some ^{15}N-labeled fertilizer and at the end of the growing season, some of this ^{15}N-labeled plant material will be deposited outside of the ^{15}N-labeled fertilizer N plot. Such lateral movement would be detectable in successive crops. The conclusion, under the growing conditions of the study by Sanchez et al. (1987), was that a ^{15}N-labeled plot size of 2 m by 2 m was adequate for determining recovery of ^{15}N-labeled fertilizer for corn under most conditions. Where lateral movement of ^{15}N-labeled fertilizer is suspected or assurance is desired, the collection of a few plant samples from known distances outside of the ^{15}N-labeled plots allows the distance of lateral movement to be evaluated.

Irrigated Corn

In a recent no-till field study with corn, Godin (1999) conducted a ^{15}N-labeled fertilizer rate by sprinkler irrigation rate study on a clay loam soil in Colorado. His microplot were 2.3 m by 1.8 m, without physical barriers. The ^{15}N-labeled fertilizer was applied using the Follett et al. method. Plant samples outside of the ^{15}N-labeled plots were collected to evaluate the lateral movement of the ^{15}N-labeled fertilizer. Results from the ^{15}N analyses of samples (whole plant) collected inside and outside of the microplots agreed with the theory described by Sanchez et al. (1987) and showed that lateral movement of fertilizer ^{15}N was negligible. The recommended fertilizer N rate resulted in significantly higher grain yields and a higher FNUE compared to higher rates of N application. Results, on the clayey soils used in this study, showed that irrigation management was not as critical to reducing N losses as was proper fertilizer N management. The study was unique in that the ^{15}N-label was observed to leach below the crop root zone (0.9 m) at the highest fertilizer N rate.

In an irrigated study with corn, Russelle et al (1981) had the objective of determining N fertilizer and water management practices that would maintain high yields on fine-textured soils of eastern Nebraska without adversely affecting the environment. During 1974 to 1976, ^{15}N-depleted ammonium sulfate was banded at rates of 112, 168, or 224 kg N ha^{-1} at planting or sidedressed at the eight-leaf growth stage. No N was applied in 1977 to 1979. In 1974 to 1977, irrigation applications of 5, 7.5, or 10 cm of water were made at 2-, 3-, or 4-week intervals, respectively, until a total of 30 cm had been applied. The plots were uniformly irrigated in 1978 to 1979. During both the treatment- and residual-years, recovery of the labeled fertilizer N in the grain was greater with sidedress N-application and with increased N-rate. Heavier, infrequent irrigation decreased labeled fertilizer-N recovery in 1974 to 1976. Maximum FNUE was obtained with low N rate, applied as a sidedressing, and with light, frequent irrigations.

^{15}N-Labeled Fertilizer Movement with Furrow Irrigation

Concepts to improve FNUE and decrease leaching for irrigated corn were investigated by Onken et al. (1979) using ^{15}N-labeled fertilizer (6.20 and 6.75 atom % ^{15}N excess). Fertilizer N as ^{15}N-labeled sodium nitrate was dissolved in water and band applied 7 cm below and 7 cm to the side of the seed at rates of 124 and 105 kg N ha^{-1} during two consecutive years. The irrigation systems investigated were sprinkler, furrow, and subirrigation. Soil samples were collected periodically during the growing season in 30-cm vertical increments through the fertilizer bands, centers of the beds, and beneath each furrow. Under sprinkler irrigation the fertilizer bands tended to move down and movement out of the surface 30 cm was faster with somewhat less lateral movement close to the soil surface than with furrow or subirrigation. Under furrow irrigation the fertilizer N tended to move toward the center of the bed and then move downward. With subirrigation the fertilizer N moved upward and outward towards the furrows and them moved downward, but remained in the upper 30 cm longer than with sprinkler or furrow irrigation.

In a more recent study, Benjamin et al. (1998) applied ^{15}N-enriched ammonium sulfate (5.0 atom % ^{15}N) on a clay loam soil in Colorado, the ^{15}N-enriched fertilizer was applied so that it was either spatially separated or not separated from the furrow in which irrigation water was applied. Nitrogen uptake and leaching were determined with alternate-furrow and every-furrow irrigation water applications, each with ^{15}N-labeled fertilizer placed either in the row or in the furrow. There were no statistical differences for the irrigation water placement effects on plant biomass or total N uptake. However, there were statistical differences in the amount of ^{15}N-labeled fertilizer leached, thus indicating that alternate-furrow irrigation is not detrimental to crop production compared to every-furrow irrigation but that its use can decrease leaching losses of fertilizer N. In a companion study, Benjamin et al. (1997) used ^{15}N-depleted ammonium sulfate (99.99 atom % ^{14}N) and determined that in-row placement of N fertilizer is beneficial for both alternate-furrow and every-furrow irrigation applications. The N placed in the rows was observed to be more available than when it was placed in the non-irrigated furrow. Non-irrigated furrow placement likely decreased N availability because of the drier soil conditions in that furrow.

^{15}N-Labeled Fertilizer Recovery by Irrigated Onions (*Allium cepa* L.)

The application rates of N-fertilizers on high-value crops, such as onions, are often much higher than are the amounts of N taken up. Also, the root systems are shallow and the potential for low FNUE and losses of N to the environment is high. Use of appropriate ^{15}N-labeled fertilizer research techniques can help identify soil and crop management practices whereby FNUE can be improved and the potential for environmental problems decreased. The N-fertilization rates normally used for onions range from 100 to 300 kg N ha^{-1}, but maximum total-N uptake (tops plus bulbs) was observed by Halvorson et al. (1998) to be only 82 kg N ha^{-1}. The accumulation of residual N below the crop root zone of irrigated onions results in a high NO_3^--N leaching potential and possible ground water contamination. The study by Halvorson et al. (1998) was designed to use producer practices, with the onions grown in 46 cm rows on raised beds and within row spacing of 7 to 10 cm. Irrigation furrows were spaced 112 cm apart on each side of the bed. A fertilizer rate of 224 kg N ha^{-1} was applied as KNO_3 in a split application with the first one-half applied in mid-May and the last one-half applied in late June. The $K^{15}NO_3$ (10 atom % ^{15}N excess) was applied to each split, but to separate microplots so that the ^{15}N uptake by the onions and the fate of the ^{15}N-labeled fertilizer applied in each split could be traced. The ^{15}N fertilizer was applied in bands using the Follett et al. method. The N was banded on the edge of the raised bed approximately midway between the bottom of the irrigation furrow and the top of the raised bed. A hoe was used to manually create a furrow in the soil into which the fertilizer N was sprayed and then immediately covered with soil to simulate a fertilizer band. Plant samples were collected inside and outside of the ^{15}N microplot areas to detect potential lateral movement problems and to compute relative fraction (RF) of plant ^{15}N inside and outside of the microplots using Equation 1 (Sanchez et al. 1987; Follett et al. 1995).

$$RF = Fx/F1 \tag{1}$$

Where Fx is fraction of labeled fertilizer in plant samples collected outside of the microplot, either in the next adjacent row within the adjacent bed or outside of the microplot in the onion rows on each end of the microplot, and F1 is the fraction of labeled fertilizer in plants samples collected within and near the center of the microplot. Thus, a value for RF of 1.0 would be obtained for plants collected within the microplot where no lateral movement of N from outside of the microplot has occurred and a value for RF of 0.0 would be obtained for plants growing outside of the microplot where there has been no lateral movement of ^{15}N from inside of an adjacent microplot. The RF for the adjacent onion row in the adjacent beds ranged from 0.0025 to 0.0110 with an overall mean of 0.0229. Thus, there was essentially no lateral movement across the irrigation furrow between the beds. The ^{15}N was expected to move laterally as a result of cultural practices and irrigation along the rows beyond the microplot ends. By final harvest, average maximum lateral detectable distance that the ^{15}N fertilizer had moved beyond the microplot boundary (where RF was equal to 0.0 based upon regression analysis) was 41 cm and 33 cm for the first and second split applications of ^{15}N fertilizer, respectively. Preliminary results from this study indicate that the FNUE was less than 20%, that there is substantial potential for growers to improve FNUE by delaying N application (possibly by using slow release fertilizer), and possibly by using several small split-N applications during the period of maximum growth and N uptake by the crop.

^{15}N Labeled Fertilizer Recovery by Potato (*Solanum tuberosum* L.)

Potatoes traditionally have their N requirement applied as pre-plant or at the time of planting. However, many growers now apply a portion of the N requirement during crop growth with the irrigation water. Westermann et al. (1988) applied ^{15}N-labeled fertilizer at different times to determine its recovery, partitioning, and translocation for Russet Burbank potatoes. They found that N recovery efficiency was 60% for pre-plant application, and over 80% and near 60% for N application during tuber growth in 1978 and 1980, respectively. The labeled N was initially concentrated in the stems and leaves, particularly if applied during tuber growth and that over 80% of the assimilated ^{15}N fertilizer was located in the tubers at the beginning of plant maturation.

Irrigated Sugarbeets (*Beta vulgaris* L.)

Evaluation of the patterns and time of nitrate uptake for sugarbeets was reported by Anderson et al. (1972). Their objective was to determine the depth to which sugarbeet roots extract nitrate-N and to use the results to determine the best soil sampling depth for the nitrate-N soil test. Two sites were selected, both on fine sandy loam soils. Sugarbeets were planted by the growers in 56 cm rows and thinned to 25 cm spacing within the row. Plot size was 3.35 by 3.35 m, within which borings to depths of either 15, 45, 75, 105, or 135 cm were made. Twenty-five ml of dilute $K^{15}NO_3$ solution (16.7 mg ^{15}N) followed by a rinse of 25 ml of water were added through a glass tube inserted full length into each hole and the holes refilled with soil. All cultivation and irrigation operations were done by the grower at each site and therefore the researchers had no control of irrigation timing or amount. ^{15}N atom % excess was measured in the petioles to use as an index of N uptake from the various profile depths and also to establish nitrate-N extraction patterns with time over the season. It was observed that sugarbeets can effectively extract nitrate-N from depths greater than 135 cm. Extraction patterns of the ^{15}N over time were related to the level of residual soil nitrate-N. A high level of residual soil nitrate-N delayed the uptake of ^{15}N from the lower profile and uptake had not occurred from that depth by the October 10 sampling. Where a medium level of residual soil nitrate-N was found, there was a more substantial uptake of ^{15}N from the 135 cm depth, even by August 8. It was concluded that the soil profile should be sampled to a depth of at least 150 cm for a residual nitrate-N soil test.

Hills, et al. (1978) reported on field studies with sugarbeets in California. Depleted ^{15}N as ammonium sulfate (0.003 % ^{15}N) was used on Reiff and Zamora loam soils, both well drained. The sugarbeets were grown in rows spaced 76 cm apart on raised planting beds. Plots were four rows wide by 15.2 m long. The fertilizer-N in granular form was applied using a custom built applicator designed to deliver granular material with an accuracy of ±5%. Fertilizer applied at planting and thinning was banded at about furrow depth and spaced 24 cm on each side of the row. Fertilizer applied at layby was placed just below the soil surface in the center of every other irrigation furrow. Fertilizer delivery by the applicator was stopped for 4.6 m of each plot, but the banding tools were left in the soil to mark the area of placement of labeled fertilizer. Within these 4.6 m long areas, the soil was removed by hoe along the furrow marks to the proper depth and ^{15}N depleted ammonium sulfate applied in one liter of water with a hand held pressurized sprayer to each marked location and the soil replaced immediately. Furrow irrigation was at 2-week intervals from mid-May through early September. Fertilizer N recovery was 47% when 112 kg N ha^{-1} were applied to achieve maximum sugar yield. The authors concluded that sugarbeets, carefully fertilized, have the potential to alleviate nitrate pollution of ground water.

LABELED FERTILIZER N STUDIES WITH CROPPING SEQUENCES

Fertilizer N Recovery

Porter et al. (1996) used labeled ^{15}N to research the uptake of fertilizer N and indigenous soil N by each crop of a no-till wheat- sorghum (*Sorghum vulgare* L.)-fallow -wheat dryland cropping sequence. They also researched the carryover of fertilizer N and the mineralization of labeled wheat residue on the ^{15}N uptake by subsequent crops. The ^{15}N fertilizer was applied using the Follett, et al. method. In this four-year no-till cropping sequence, they determined that N-uptake by the plants transferred most of the fertilizer N to aboveground biomass. Crop residue deposition, immobilization, and mineralization maintained the fertilizer N in the top 60 cm of soil. At the end of the four yr cropping sequence 90 and 87% of applied fertilizer N was accounted for at the 56 and 112 kg N ha^{-1} rates, respectively. Of this N, 24 to 28% remained in the soil at the end of 4 yrs. The 10- to 13% of the applied fertilizer N that was unaccounted for was assumed to have been lost by denitrification or NH_3 volatilization.

Use of ^{15}N to Study Crop Residue/Tillage Management Effects on Soil-N Dynamics

On a Vertisol

Vertisols have high clay content; pronounced volume change with moisture change; deep wide cracks at some season; and evidences of soil movement in the form of slickensides, gilgai micro-relief, and wedge shaped structural aggregates. In Mexico, vertisols occupy about one million ha. Current practice is to produce two crops yr^{-1} under irrigation, burn the residue of both crops, and plow the soils deeply and frequently. Soil organic matter loss is considered to have seriously altered the soil N dynamics and N cycling capacity of these soils, and their crop productivity has decreased. Castellanos et al. (1998) designed the study using rates of ^{15}N labeled fertilizer, along with the return of crop residues and various tillage systems, to help evaluate the rebuilding of soil organic carbon (SOC) and soil fertility and to evaluate the N dynamics of the system. Treatments were three ^{15}N-labeled fertilizer rates (0, 150, and 300 kg N ha^{-1}) and five tillage treatments. Micro-plot size was 1.8 m by 2.8 m and the fate of the ^{15}N fertilizer was followed through several crop cycles. The ^{15}N fertilizer was applied using the Follett et al. method. After 2 yrs (4 successive crops), treatment differences in amount of SOC were significant but only to the 0-to 15 cm depth. Highest SOC was observed for the wheat-corn (no-till) treatment (Fig. 4). Amount of microbial- biomass C and N was highest where the most residue C was returned. Highest grain yields and ^{15}N-labeled fertilizer recovery was for wheat planted after beans and the lowest grain yields was for wheat planted after corn. Treatments with large amounts of crop residues had lower crop-^{15}N recoveries, especially when residues remained on the soil surface. N immobilization occurs at the beginning of the season and low rates of fertilizer N have minimum benefit to crop yield. With low rates of N fertilization, residue burning produced higher yields than did conventional and conservation tillage. At the highest N rates, differences in grain yield between tillage/residue management treatments were not significant ($P<0.05$). The results indicate that residue management has a large effect on N availability to the crop and influences soil carbon sequestration.

Small Grain Potato Rotation

Potatoes can be grown in a 2 yr cropping sequence with small grains such as wheat and barley (*Hordeum vulgare* L.). There is interest by farmers in south central Colorado to improve FNUE and irrigation water use efficiency (San Luis Valley Water Quality Demonstration Project 1999). The question exists about the importance to the subsequent potato crop of the residual soil N and recycling of small-grain residue N. Questions also exist about the relative merits of spring-versus winter-small grains. During the first year of this study[2] spring barley, soft

[2] Personal communication with Dr. Jorge Delgado, USDA-ARS, Fort Collins, CO.

white spring wheat, and hard red winter wheat were established in 3.4 m by 1.6 m plots and fertilized with ^{15}N labeled fertilizer at the rate of 95 kg N ha^{-1}. The ^{15}N-labeled fertilizer was applied using the Follett et al. method. The technique used to minimize concerns about lateral movement of the ^{15}N label was that plots within replications were placed directly together such that the area receiving ^{15}N fertilizer was 3.4 m by 4.8 m. Following harvest of the small grain crops, their ^{15}N-labeled residues (straw plus chaff) were moved to an adjacent and identically sized and designed plot area and incorporated into the soil. Thus, the original ^{15}N fertilized area can be used to evaluate the recycling of residual soil ^{15}N plus ^{15}N-labeled root and crown material and the adjacent area can be used to evaluate the recycling of ^{15}N from the small-grain straw plus chaff. The size of each replication after relocating the straw and chaff was doubled to 3.4 m by 9.6 m, a size that readily accommodates planting and other cultural operation associated with growing the subsequent potato crop. Thus, ^{15}N-label fertilizer can be traced from its initial soil application through the small grain cropping system and into the potato crop using producer practices.

FERTILIZER ^{15}N DYNAMICS

Fertilizer N Dynamics and Soil Biology

Residual Fertilizer N

Use of ^{15}N in field studies allows multi-year analysis of the effects of cropping on the fertilizer N dynamics in soil. Broadbent (1980), using depleted ^{15}N-labeled fertilizer, measured residual effects while continuing a constant rate of N-application. Rates of N application were 0, 90, 180, and 360 kg N ha^{-1}. Plot boundaries were shifted each year so that part of the plot area, which Broadbent designated as Area I, received labeled fertilizer N each year, but with a history of unlabeled fertilizer at the same rate in previous years. Part of the plot area, designated Area II, received labeled fertilizer each year so that cumulative effects were measurable, and a third part, designated Area III, received labeled fertilizer the preceding year and conventional fertilizer during the current year. Estimates of residual effects were obtained from the isotopic composition of corn grown each year in the various areas. After five consecutive crops of corn, with the same rate of fertilizer applied each year, a final crop of sorghum was grown with no application of fertilizer. It was concluded that with efficient management which avoids over fertilization, the residual N remaining is not sufficient to affect application rates. Substantial quantities of residual N reflected over fertilization and/or insufficient irrigation water in previous years.

Microbial Biomass and N Cycling

Multi-year analysis can allow for consideration of the influence of N that is recycled from the crop residues of the previous crop into various soil N pools. Follett et al. (1995) evaluated soil C and N pools in a no-till wheat- sorghum-fallow- wheat dryland cropping sequence under two fertilizer N rates on a loam soil near Akron, Colorado. The ^{15}N fertilizer was applied using the Follett, et al. method. Besides measuring total soil N and ^{15}N, microbial biomass C, N, and ^{15}N were determined by incubating soil subsamples. Results showed that, in the top 122 cm of the soil profile, the soil N derived from fertilizer (Ndff) that remained in the soil became increasingly concentrated in the topsoil. Between 50 and 70% of the total amount of Ndff in the top 122 cm of soil was found in the top 10 cm and of that amount about 30% of the Ndff in the top 10 cm was in microbial biomass during the third and fourth yrs. The amount of Ndff that was in the microbial-biomass soil fraction stabilized during the last two years and remained quite constant. It was determined that under no-till management, biological processes are instrumental to the conservation of soil Ndff from losses out of the soil-plant system.

Soil ^{15}N-Dynamics of Bromegrass (*Bromus inermis* Leyss) and Switchgrass (*Panicum virgatum* L.)

The dynamics and changes in soil-N pools under non-fertilized and fertilized bromegrass sod compared with an area of non-fertilized and fertilized sod that was killed and reseeded to switchgrass required the use of 98 atom % ^{15}N tracer. The fertilizer rates were 0 and 80 kg N ha^{-1} applied annually. Tracer ^{15}N was added to all microplots at the rate of 8 kg N ha^{-1}, thus resulting in actual rates for the first year of the study of 8 and 88 kg N ha^{-1}. In subsequent years only normal abundance fertilizer material is applied for the 80 kg N ha^{-1} rate and ^{15}N tracer will not be reapplied. Allowing that a small fertilizer N effect may occur during the first year on the "0-N" rate plots, this procedure allows ^{15}N tracer to be put into the soil system. The ^{15}N fertilizer was applied using the Follett et al. method. The study was designed to apply the ^{15}N tracer to a larger microplot area (Fig. 3), that could then have the soil within each microplot sampled up to ten times (see the small white board in Figure 3) while maintaining about a 30 cm border around all sides of each small sub-sampling area within each microplot. Normal cultural practices can be used and sampling and follow up studies can be planned for several future growing seasons.

SUMMARY AND CONCLUSIONS

The use of ^{15}N-labeled fertilizer techniques offers many opportunities to improve and refine the knowledge required to improve FNUE and to better understand the dynamics of the N cycle under agricultural systems. The examples reported in this paper illustrate that there is no single best technique to use ^{15}N-labeled fertilizer in field experiments. Rather the theme of this paper has been that with creativity and careful planning, use of ^{15}N-enriched or -depleted fertilizers help researchers meet their objectives in numerous types of experiments, for many crops and cropping systems, in many types of agroecosystems, and on many soil types. The goal has been to provide a wide enough range of examples for scientists to draw upon and then, using their own experience and creativity, for them to design studies that can accomplish the objectives for their own research program.

Examples of microplot studies have been included where physical barriers were used and where no physical barriers were used. Some of the strengths and limitations of each approach were discussed. Even though ^{15}N-labeled fertilizers offer considerable opportunity to understand N cycling, there are limitations. Types of ^{15}N-labeled fertilizer materials have been discussed including some precautions about the use of ammonium forms versus nitrate forms. Literature sources for additional reading about the use of ^{15}N tracer technology are provided. This further reading is recommended to scientists who have not used ^{15}N tracers previously because there are many issues related to sample -handling, -quality assurance, and -quality control that are required to obtain good data and avoid the contamination and/or loss of experimental samples.

The examples of studies that have been discussed illustrate that with careful planning and imaginative approaches, important questions can be addressed. These questions can be addressed not only in the laboratory or greenhouse studies, but can be effectively addressed in field-experimental plots and in farmers' fields. Artificial systems or simulated cultural practices do not need to be used unless desired. In summary, technique to conduct ^{15}N-labeled fertilizer studies are possible where traditional and/or innovative cultural practices are used, including for grazinglands, mountain meadows, field crops, vegetable crops, within various cropping sequences, and for irrigated and non-irrigated systems. Careful evaluation of whether an ^{15}N-labeled fertilizer study is important enough to do is very necessary because such studies can be expensive and often require significant amounts of time and labor.

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