# Border Effects on Nitrogen15 Fertilized Winter Wheat Microplots Grown in the Great Plains

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# SOILS

## Border Effects on Nitrogen-15 Fertilized Winter Wheat Microplots Grown in the Great Plains

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### ABSTRACT

**Field experiments requiring $^{15}N$ enriched fertilizer are costly, thus microplot techniques are generally used. Placing physical barriers around microplots to contain the $^{15}N$ may introduce artifacts that affect N recovery by crops, limit types and numbers of measurements, and cause other restrictions. The purpose of this experiment was to determine minimum microplot size (without the use of barriers) for accurately measuring enriched $^{15}N$ uptake into a winter wheat crop, while using normal field cultural practices. Winter wheat (*Triticum aestivum* L.) was seeded into $KNO_3$ fertilized (56 and 112 kg N $ha^{-1}$) plots (4.57 m by 3.05 m) on a Platner silt loam soil (fine montmorillonitic mesic Aridic Paleustol). Within each larger plot, four microplots (2.29 m by 1.83 m) were fertilized with 10 atom % $^{15}N$ enriched $KNO_3$ at the same rate as for main plots. Nitrogen-rate treatments were replicated four times in a randomized block design. Above ground plant material was harvested (0.3 m of row) from six adjacent rows at flowering (Feeke's scale = 10.5). Three rows were harvested from inside ($^{15}N$ enriched $KNO_3$ added) and three from outside ($^{15}N$ enriched $KNO_3$ not added) of the microplots. Plant uptake of total N into plant tops was not significantly different across any of the six harvested rows. Dry matter yields and total-N uptake were significantly larger for the 112 than for the 56 kg N $ha^{-1}$ fertilizer rate, as were the $^{15}N$ uptake and atom $^{15}N$ % values in plant material within the microplots. In rows adjacent to microplot borders, concentrations of $^{15}N$ in plant material changed rapidly; but there were no differences beyond 0.46 m inside or outside the microplots. These results indicate that minimum microplot size for studies with fall-applied $^{15}N$ on winter wheat grown in the Great Plains is 1.5 by 1.5 m.**


NITROGEN TRACER techniques provide a method for making quantitative measurements of N-transformation processes. In addition, tracer methods permit added fertilizer N to be distinguished from indigenous or fertilizer N. Field experiments that have utilized $^{15}N$ enriched fertilizer N have varied in type from those using only single plants, through small one row plots, microplots, and lysimeters.

Because highly enriched $^{15}N$ is considered too expensive for use on field plot experiments, investigators have used depleted $^{15}N$ materials to measure plant recovery and movement of fertilizer-N for such experiments (Broadbent and Carlton, 1978). However, lack of isotope enrichment in depleted $^{15}N$ studies usually prevents detailed examination of N-transformations, such as immobilization or mineralization. Large plots impose additional limitations such as: spatial variability; the need for large equipment and coordination of field efforts for planting, fertilizing, pest control, and harvesting; and, increased labor needs. However, much can be learned from field plots concerning the influence of environmental and soil factors on movement of N in soil, plant uptake of mineral N, the influence of tillage and residue management on organic N transformations, and gaseous N losses.

Physical barriers buried around small plots to isolate them from the surrounding soil can be used for conducting $^{15}N$ field studies. The barriers, often cylinders made of steel, are driven into the soil to delineate boundaries for the small microplot. A single cylinder, approximately 30 cm in diameter pressed into the soil to a depth of 45 to 60 cm, was reported to constitute a satisfactory plot and was reliable enough to determine small changes in fertilizer $^{15}N$ balance of soil (Carter, et al., 1967). This microplot procedure has many advantages. Mobile forms of N are restricted to vertical movement and lateral movement of N out of or into the experimental area is prevented. Water erosion is prevented and wind erosion is minimized. Cylinders allow test plants to absorb N only from the experimental area, and prevent tagged fertilizer from being taken up by outside plants. Well controled conditions are provided while realistic results under field conditions are achieved (Myers and Paul, 1971).

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Although barriers eliminate problems associated with lateral movement, they themselves can introduce artifacts affecting fertilizer recovery by crops. These artifacts may result from: an inability to perform normal tillage practices, inability of root systems to achieve normal distribution and size, creation of artificial pores that increase aeration or movement of water and solutes, disruption of macropore systems that may influence aeration or movement of water and solutes (Sanchez, et al., 1987), and concentrated water-infiltration down the cylinders walls, particularly in cracking clay soils. Additional disadvantages of using steel cylinders are: soil temperatures may be affected by heat conducted into the soil by the steel cylinder (this effect is generally considered small as a result of the volume of soil contained in the cylinder); possible soil compaction during cylinder installation (Myers and Paul, 1971); and space limitations for experimenting with crops such as corn (*Zea mays* L.), sorghum [*Sorghum bicolor* (L.) Moench], and potatoes (*Solanum tuberosum* L.) where each cylinder will likely only contain one plant at normal row spacings.

An alternative to the use of physical barriers around very small microplots is to utilize larger microplots without barriers. Such an approach has been studied for corn (Jokela and Randall, 1987; Sanchez, et al., 1987; Stumpe, et al., 1989). The purpose of this experiment was to determine minimum microplot size, without barriers, for measuring enriched $^{15}$N-isotope uptake by winter wheat in the Great Plains, while allowing normal cultural practices. To do so requires that all roots of plants being measured are grown in soil in which $^{15}$N fertilizer distribution is the same as it would be in a large treated area. Two factors influencing the area required are lateral root distribution and lateral movement of fertilizer N from the treated area (Olson, 1980). It is necessary to identify how small a plot must be before lateral movement or lateral root growth introduces significant errors. Lateral movement of $^{15}$N occurs by mass flow or diffusion in soils, by physical movement during cultural operations, or through movement by wind/or water, and by translocation in plant tissues (Sanchez, et al., 1987).

## METHODS AND MATERIALS

Winter wheat ('TAM *107*') was seeded in September 1987 at 0.30-m row spacing at 60 kg seed ha$^{-1}$ with a deep furrow drill into Platner loam soil on a level to nearly level site (< 1% slope) near Akron, CO. The field had been chemically fallowed under no-till for 14 mo after a previous wheat crop. Total soil N and C were determined by automated combustion analyses procedures (Nelson and Sommers, 1982; Marshall and Whitehead, 1985). Soil N and C in the 0- to 10-cm and 10- to 30-cm depths contained 6.51 and 6.10 g total C kg$^{-1}$ soil and 0.80 and 0.75 g total-N kg$^{-1}$ of soil, respectively (Table 1). Inorganic C was not removed prior to total C analysis since measured levels (Nelson and Sommers, 1982) were less than 1 and 2 g kg$^{-1}$ in the 0- to 10- and 10- to 30-cm depths, respectively. Texture of the soil was a loam in the 0- to 10-cm depth and a sandy clay loam in the 10- to 30-cm depth. Other selected chemical characteristics are shown in Table 1. The soil does not possess any salinity/sodicity problems (USDA, 1954; Workman, et al., 1988).

Phosphorus fertilizer was banded below the seed at planting at the rate of 11.2 kg P ha$^{-1}$ as superphosphate. Available P (Table 1) measured the following spring (Soltanpour, et al. 1982; Workman, et al., 1988) indicated that soil P was sufficient and not restricting crop yield.

**Table 1. Characteristics of Planter loam (composite of 16 cores).**

| Soil analyses | Depth (cm) 0–10 | 10–30 |
|---|---|---|
| Total N (g kg$^{-1}$) | 0.80 | 0.75 |
| Total C (g kg$^{-1}$) | 6.51 | 6.10 |
| Paste pH | 6.8 | 7.4 |
| Sodium adsoption ratio | 1.5 | 1.0 |
| Electrical conductivity (dS m$^{-1}$) | 1.0 | 0.6 |
| Extract. P (mg kg$^{-1}$) | 14.4 | 6.7 |
| CEC (cmol. kg$^{-1}$) | 121.0 | 177.0 |
| Exch. Ca (g kg$^{-1}$) | 1.060 | 1.860 |
| Exch. Mg (g kg$^{-1}$) | 0.259 | 0.473 |
| Exch. K (g kg$^{-1}$) | 0.617 | 0.499 |
| Exch. Na (g kg$^{-1}$) | 0.020 | 0.029 |

The study reported here is part of a larger study consisting of a randomized block design of three N rates (0, 56 and 112 kg N ha$^{-1}$ as $KNO_3$) with four replications. Main (N rate) plots were 9.14 by 12.19 m and within each main plot were eight subplots (4.57 by 3.05 m). Microplots (2.29 by 1.83 m) were established within four of the subplots in each main plot and were randomly chosen to receive 10.3730 atom % enriched $KNO_3$ at the same rate of N fertilization as was received by the main- and sub-plots that they were located within.

Nitrogen (unlabeled fertilizer $KNO_3$ in granular form) was broadcast on main plots on 8 September immediately after planting. Microplots that were to receive $^{15}$N enriched $KNO_3$ were covered with plastic sheets to catch all applied fertilizer. After the plastic sheets and the unlabeled fertilizer $KNO_3$ that fell onto them were removed, $^{15}$N enriched $KNO_3$, dissolved in 1.4 L of water, was sprayed onto the soil surface of the microplots at the desired rate of N application using a hand-held sprayer maintained at a constant pressure of 0.14 MPa. Precipitation between planting and the 6 June harvest was about 30 cm, with about 14 cm falling after 1 May. Precipitation before May came either as snow or accounted for 2 cm or less of rainfall. In addition, no single rainfall event exceeded 1.3 cm prior to 1 May, after which the largest rainfall event was 4.0 cm on 19 May.

Above-ground plant material was harvested about 3 cm above the ground surface from 0.3 m of row on 6 June 1988 by clipping from six adjacent rows (Fig. 1) on the microplot border and at a distance of 0.46 m to 0.76 m from the end of $^{15}$N-fertilized microplots. The plant material was harvested when wheat was at flowering (Feekes scale = 10.5)

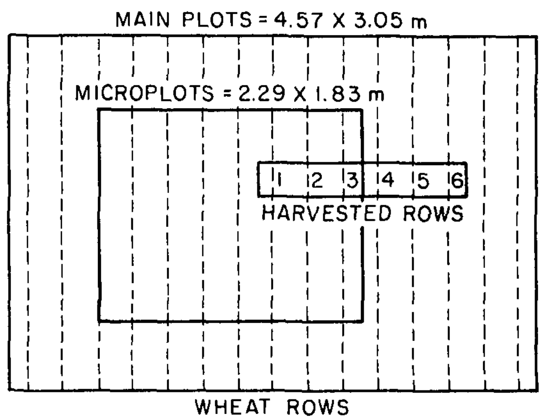


Fig. 1. Layout of $^{15}$N fertilized microplots inside non-$^{15}$N fertilized subplots.

(Large, 1954). The three rows harvested from inside the microplots had received $^{15}N$ enriched $KNO_3$ while the three adjacent rows harvested from outside the microplots had received unlabeled fertilizer $KNO_3$. Plant material from each row was oven-dried (65 °C) and finely ground to pass through a 150 μm sieve. Total N and $^{15}N$ content were determined by automated combustion-isotope ratio mass spectrometry (IRMS) using a VG-903 IRMS (VG Isogas, Middlewich, England) coupled by a Europa Scientific Interface (Europa Scientific Ltd., Crewe, England) to a Carlo Erba C/N analyzer (Haake Buchler Instruments Inc., Saddle Brook, NJ)[1] (Marshall and Whitehead, 1985). Statistics were determined using analysis of variance procedures (SAS, 1985).

## RESULTS AND DISCUSSION

### Fertilizer Application

Above ground yields and total-N uptake across the six rows (Fig. 1) were the criterion used to determine rate and uniformity of application of $^{15}N$ enriched fertilizer inside the microplots compared to that of unlabeled fertilizer N applied outside of the microplots (Table 2). Mean dry matter yields across the six rows (three inside and the three outside of the microplots) were not significantly different. However, N-fertilization rate resulted in a significant yield increase. There was no significant interaction on dry matter yields between harvested row position and N-fertilization rate nor was there any significant difference in total-N uptake across Rows 1 through 6 (Table 2), yet a significant increase in the mean total-N uptake did result from increased rate of N fertilization. No significant interaction existed between harvested row position and N-fertilization rate for total-N uptake. These results show that plant responses were the same on the inside as they were on the outside of the microplots, even though the 15N enriched fertilizer was sprayed onto the microplots and unlabeled fertilizer N was broadcast, in granular form, outside the microplots.

### Fertilizer Nitrogen-15

We assumed that the area required for lateral root distribution would be the primary factor influencing required microplot size. Over-winter movement of fertilizer $^{15}N$ from the treated area by wind and water erosion was likely minimized by the presence of the growing winter wheat crop. Rainfall amounts for the period between planting and harvest were either small during any single event or came after the vegetative development of the crop was great enough to minimize or prevent surface water runoff or soil erosion. The only soil disturbing cultural operation performed was seeding of the wheat into the fertilized soil with a deep furrow drill.

The fraction ($F$) of total N uptake derived from $^{15}N$ enriched fertilizer was calculated using Eq. [1] (Sanchez, et al., 1987)

$$F = \frac{\mathrm{As} - \mathrm{Ar}}{\mathrm{Af} - \mathrm{Ar}} \qquad [1]$$

Where: As is the atom % $^{15}N$ measured in the harvested plant sample, Af is 10.3730 atom % $^{15}N$ (measured in the $^{15}N$ enriched fertilizer), and Ar is the atom % $^{15}N$ of the reference harvested plant material from non-$^{15}N$-enriched fertilizer treatments. Measured values of Ar were 0.3861 and 0.3822 atom % for the 56 and 112 kg N $ha^{-1}$ of unlabeled fertilizer N treatments, respectively. Rate of N-fertilization did not result in values of Ar that were significantly different. Unlabeled fertilizer N was assumed to have the same atom % $^{15}N$ as that measured for Ar.

Uptake of $^{15}N$-enriched fertilizer N (Ef) into the above ground dry plant material (Table 3) was then calculated from the amount of total N-uptake (Table 2) by using Eq. [2].

$$\mathrm{Ef} = F \times \text{total N-uptake} \qquad [2]$$

Analysis of variance shows there was no significant difference in Ef between Rows 1 and 2 inside of the microplots or between Rows 5 and 6 outside of the microplots. Ef decreased most rapidly at the microplot border between Rows 3 and 4. Atom % $^{15}N$ in the harvested plant material (Table 3) showed the same relationship to row position as did Ef. Significant interaction of N-fertilizer rate and row position resulted from a significant increase of Ef and atom % $^{15}N$ with

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

**Table 2. Effect of row position, relative to the microplot border, on dry-matter yield and total-N uptake of winter-wheat at flowering.**

| Distance† from plot border | Row number | Dry matter 56 | Dry matter 112 | Dry matter Mean‡ | Total-N uptake 56 | Total-N uptake 112 | Total-N uptake Mean‡ |
|---|---|---|---|---|---|---|---|
| | | N fertilization rate (kg N $ha^{-1}$) | | | | | |
| m | | kg $ha^{-1}$ | | | | | |
| −0.76 | 1 | 8198 | 8605 | 8401a | 102 | 107 | 104a |
| −0.46 | 2 | 8087 | 9180 | 8633a | 95 | 112 | 103a |
| −0.15 | 3 | 7849 | 8868 | 8359a | 96 | 112 | 104a |
| +0.15 | 4 | 7580 | 9607 | 8594a | 90 | 117 | 104a |
| +0.46 | 5 | 8084 | 8474 | 8279a | 97 | 111 | 104a |
| +0.76 | 6 | 7826 | 8770 | 8298a | 90 | 116 | 103a |
| Mean‡ | | 7937b | 8917a | | 95b | 112a | |

† Negative (−) and positive (+) numbers indicate distance of wheat-rows inside and outside of the $^{15}N$-fertilized microplot border, respectively.
‡ Means with the same letter are not significantly different at the $P = 0.05$ level of probability.

**Table 3. Effect of row position, relative to the microplot border, on winter-wheat uptake of $^{15}N$ enriched fertilizer–N and atom % $^{15}N$ in above-ground dry-matter at flowering.**

| Distance† from plot border | Row number | $^{15}N$-enriched Fertilizer-N uptake 56 | 112 | Mean‡ | Atom % $^{15}N$ in plant material 56 | 112 | Mean‡ |
|---|---|---|---|---|---|---|---|
| | | N-fertilization rate (kg N $ha^{-1}$) | | | | | |
| m | | kg $ha^{-1}$ | | | atom % | | |
| −0.76 | 1 | 32 | 45 | 38a | 3.4514 | 4.6177 | 4.0346a |
| −0.46 | 2 | 29 | 47 | 38a | 3.3811 | 4.5750 | 3.9780a |
| −0.15 | 3 | 22 | 38 | 30b | 2.5914 | 3.7361 | 3.1638b |
| +0.15 | 4 | 7 | 15 | 11c | 1.1365 | 1.6515 | 1.3940c |
| +0.46 | 5 | 0 | 1 | 1d | 0.4028 | 0.5063 | 0.4546d |
| +0.76 | 6 | 0 | 0 | 0d | 0.3926 | 0.4135 | 0.4030d |
| Mean‡ | | 15b | 24a | | 1.8815b | 2.5834a | |

† Negative (−) and positive (+) numbers indicate distance of wheat-rows inside and outside of the $^{15}N$-fertilized microplot border, respectively.
‡ Means with the same letter are not significantly different at the $P = 0.05$ level of probability.

increased application rate of $^{15}$N-enriched fertilizer inside of the microplots. Because $^{15}$N-enriched fertilizer was not applied outside of the microplots, both Ef and atom% $^{15}$N in plant material decreased toward zero and natural abundance levels, respectively, for row Positions 4 through 6.

Comparison of Row 6 data for the 56 and 112 kg N ha$^{-1}$ fertilized treatments showed slightly elevated levels of atom % $^{15}$N (Table 3) compared to the non-$^{15}$N treatments fertilized at corresponding rates (Ar). Atom % $^{15}$N values for Row 6 and the check treatment values (Ar) were not significantly different at either N-fertilization rate. However, the slightly elevated levels of atom % $^{15}$N observed for Row 6 may indicate that plant roots were still taking up negligible amounts of $^{15}$N from the microplot areas even at a distance of 0.76 m.

### Symmetry about microplot borders

In theory, plants positioned exactly on the border of a microplot (i.e. exactly half-way between Rows 3 and 4 in Fig. 1) should take half of their N from within the microplot (fertilized with labeled N) and the other half from outside of the microplot (fertilized with unlabeled N). Thus, with no lateral movement of either the labeled or unlabeled fertilizer N, isotope enrichment of plant tissue as a function of row position should be symmetrical about the microplot border. The relative fraction (RF) of plant N (based upon the calculation of $F$ as shown by Eq. 1) derived from labeled fertilizer at the various row positions on each side of the microplot border was calculated using Eq. 3 (Sanchez, et al. 1987).

$$RF = Fx/Fl \qquad [3]$$

Where $F$x is fraction of labeled fertilizer in plant samples collected from Rows 1 through 6, and $F$l is fraction of labeled fertilizer in plant samples collected from Row 1 (Fig. 1). As shown in Fig. 2, RF for both 56 and 112 kg N ha$^{-1}$ fertilizer rates are about 0.5 at the midpoint between Rows 3 and 4 (the microplot border). This observation is consistent with a symmetrical plant N-uptake pattern as described above.

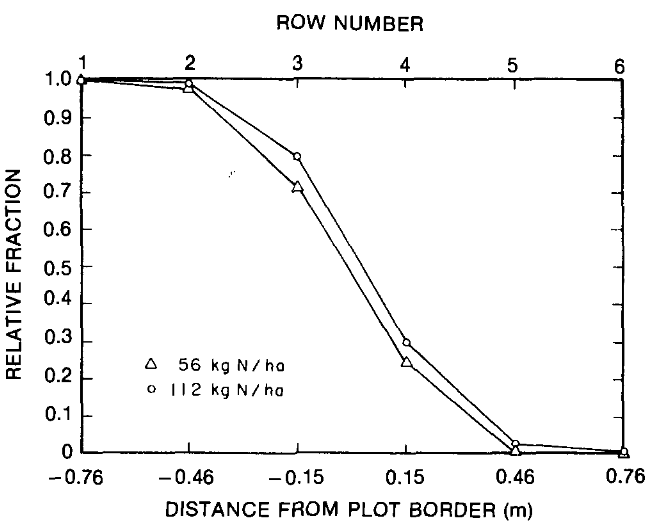


**Fig. 2. Effect of row position, relative to the microplot border, upon relative fraction of $^{15}$N enrichment in above-ground dry-matter of winter-wheat at flowering.**

Values for RF range from 1.0 within the microplot to 0.5 at the microplot border and then to 0.0 at Row 6 outside of the microplot border. These values represent the fraction of fertilizer-N uptake from the labeled fertilizer. Therefore, (1.0 − RF) should represent the fraction of fertilizer-N uptake from unlabeled fertilizer N and ranges from 0.0 for Row 1, within the microplot, to 0.5 at the microplot border and then to 1.0 at Row 6 (i.e. 1.0 − RF is a mirror image of RF, when graphed.)

Symmetry was tested by comparing RF for Rows 1 to 3 (within the microplots) to 1.0 − RF for Rows 4 to 6 (outside of the microplots). This comparison is based upon the premise that relative fraction of unlabeled fertilizer-N uptake (1.0 − RF) in the rows adjacent to but outside of the microplot border is a mirror image (MI) of relative fraction of labeled fertilizer-N uptake (RF) in the rows adjacent to but inside of the microplot border. Analysis of variance procedures (SAS, 1985) were used to test for symmetry and showed significance ($P$ = 0.0001) only for distance from the microplot border. No significant differences were observed for MI, N − rate, MI × N − rate, MI × distance, or for MI × N − rate × distance. Therefore, we conclude that N-uptake from both the labeled and non-labeled fertilizer-N was symmetrical about the microplot border for both rates of N-fertilization and that lateral movement of labeled fertilizer-N outside of the microplot borders was insignificant under the conditions of this study,.

## CONCLUSIONS

Use of $^{15}$N-labeled fertilizer in microplots without barriers is feasible in field studies as long as minimum microplot size and its management meet certain criteria. These are that microplots be large enough that all roots of plants being measured are grown in soil in which $^{15}$N-fertilizer distribution is the same as it would be in a large treated area (Olson, 1980). If lateral movement of fertilizer-N from or root distribution outside of the treated area occurs, then results will be affected. For $^{15}$N fertilized winter wheat, grown in 0.30 m spaced rows, border effects extended into the microplots about 0.50 m. Sampling rows that were 0.76 m or further inside of the microplots met the criteria that plant-$^{15}$N uptake responded as if the plants were grown in a large $^{15}$N-fertilizer treated area. Therefore, minimum plot size needed to be double the 0.76-m width or about 1.50 m by 1.50 m.

Because barriers were not used, normal cultural practices were possible. Use of plow- or disk-tillage or other soil disturbing practices will likely require larger microplots than for the no-till system used in this study. Larger microplots are likely needed, if $^{15}$N measurements extend past one crop growth cycle; especially where lateral movement of N is likely to be enhanced by harvesting, cultural practices, soil erosion, weather, or other conditions. Advantages derived by conducting $^{15}$N experiments on field plots not having barriers appear worthy of the time and care required, especially where use of small microplot size helps minimize costs associated with the use of $^{15}N$ fertilizer while eliminating costs of installing barriers around $^{15}N$-fertilized microplots.

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