Plant and Soil (2005) 272: 263–276
DOI 10.1007/s11104-004-5294-x

Soil aggregate sequestration of cover crop root and shoot-derived nitrogen

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Received 1 July 2004. Accepted in revised form 24 October 2004

Key words: aggregate concentric layers, ^{15}N, nitrate leaching, rhizosphere, rye root, rye shoot, soil aggregate

Abstract

Cover crop roots and shoots release carbon (C) and nitrogen (N) compounds *in situ* during their decomposition. Depending upon the season, these C and N compounds may be sequestered, the C may be respired or the N may be leached below the root zone. A field study was established to identify the contributions of cover crop root and shoot N to different regions within aggregates in the A_p horizon of a Kalamazoo loam soil. Fall-planted rye plants (*Secale cereale* L.) were labeled the next May with foliar applications of solutions containing 99% atom $(^{15}NH_4)_2SO_4$. Isotopic enrichment of soil aggregates ranging from 2.0 to 4.0, 4.0–6.3 and 6.3–9.5 mm across was determined following plant residue applications. Concentric layers of aggregates were removed from each aggregate by newly designed meso soil aggregate erosion (SAE) chambers. Non-uniform distributions of total N and recently derived rye N in soil macroaggregates, across time, suggested that the formations and functions of macroaggregates are very dynamics processes and soil aggregates influence where N is deposited. Early in the season, more ^{15}N migrated to the interior regions of the smallest aggregates, 2–4 mm across, but it was limited to only surfaces and transitional regions of the larger aggregates, 6.3–9.3 mm across. Exterior layers of aggregates between 6.0 and 9.5 mm retained 1.6% of the $N_{derived\ from\ roots}$ in July 1999, which was three times more than their interior regions. This was slightly greater than the % $N_{derived\ from\ shoot}$. One month later, as the maize root absorption of N increased rapidly, % $N_{derived\ from\ roots}$ and % $N_{derived\ from\ shoot}$ were nearly equal in exterior layers and interior regions of soil aggregates. This equilibrium distribution may have been from either greater diffusion of N within the aggregates and/or maize root removal form aggregate exteriors. Results supported that most of roots grew preferentially around surfaces of soil aggregates rather than through aggregates. Cover crop roots contributed as much N as cover crop shoots to the total soil N pool. Subsequent crops use N from the most easily accessible zones of soil structure, which are surfaces of larger soil aggregates. Therefore maintaining active plant roots and aggregated soil structure in the soil enhances N sequestration and maximize soil N availability. These studies suggest that the rapid and perhaps bulk flow of soil N solutions may bypass many of the central regions of soil aggregates, resulting in greater leaching losses.

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Introduction

Cover crops used to reduce leaching of NO_3^- (Ditsch et al., 1993; McCracken et al., 1994) contribute to the improvement of soil organic matter supplying residue additions in the early spring and throughout the summer. Living rye roots, decomposition and by-products associated with the rye root and shoot residues are effective contributors to soil nutrient cycling and aggregate formation and stabilization. Stable soil aggregates are important for maintaining soil structure and productivity and for enhancing soil characteristics such as water retention, gaseous diffusion, hydraulic conductivity and erodibility. Combinations of soil texture, ions, water, SOM and microbial–faunal activities determine the various degrees of aggregate stability (Tisdall, 1991). Soil organic matter, especially readily available C, is an essential cementing agent of stable structural units. In many studies positive relationships have been found between water stable aggregates and soil organic C content (Angers et al., 1997; Jastrow, 1996; Kavdır et al., 2004; Six et al., 2000). Plant roots and shoots directly contribute to soil organic mater, and thereby to soil aggregate stability, and indirectly to the stimulation of microbial activity in the soil (Angers and Mehuys, 1989).

Decomposition of organic matter and plant growth lead to development of a hierarchical aggregate structure when organic matter is the major binding agent for soil aggregates (Oades and Waters, 1991; Tisdall and Oades, 1982). Three main mechanisms of C stabilization have been proposed; biochemical stabilization, stabilization by association with silt and clay particles, and by means of physical protection within aggregate structures (Christensen, 1996). The stabilization of organic C and N within aggregates is partly related to the decreased oxygen concentration in the center of the micro- and macro-aggregates. Several studies investigating oxygen profiles across aggregates have reported steep declines in oxygen concentrations over small distances from the aggregate surface. Oxygen concentrations in interior decreased in large aggregates (Hojberg et al., 1994; Sextone et al., 1985). Aerobic respiration potential was reported to be greater near the surfaces of aggregates (Sierra and Renault, 1996). Therefore, it was assumed that oxygen gradients most likely control microbial activities associated with SOM decomposition, and C and N accumulations in the soil aggregates.

Numerous studies have been reported on the formation, stabilization, and effect of different soil and crop management systems on soil aggregation (Roberson et al., 1995; Wood et al., 1991). However, there is little information on the location of recently decomposed plant residues within soil aggregates (Angers et al., 1997). Wang et al. (2001) suggested that soil management that favors soil aggregation could increase availability of applied phosphorus (P) and that distribution of soil aggregates should be considered in making P management decisions. In another research, autoradiography of sorbed ^{32}P and P sorption studies indicated that added P was initially sorbed to a 0.188-mm layer around aggregates and remained in this peripheral layer for up to 28 days (Linquist et al., 1997). Perhaps analyzing bulk soil may supply limited information for at least P content and its availability for plant growth. Therefore, soil aggregates especially the location of plant nutrients within aggregates should be considered.

Contrasting C concentrations within interior and surface regions of aggregates were reported by Santos (1998), Smucker et al. (1997) and Chenu et al. (2001). Therefore, plant nutrition in soils is controlled in part by the availability of nutrients within specific layers or regions of soil aggregates. Clay illuviation, preferential movement of water, weathering of clay and preferential growth of roots can change the compositions of aggregate surfaces (Horn et al., 1995; Smucker et al., 1997; Whiteley and Dexter, 1983; Wilcke and Kaupenjohann, 1998).

Living roots influence the chemical and biological properties of rhizosphere soil (Fisher et al., 1989) by changing pH, redox potential, water and nutrient content of the rhizosphere. They may create rapid wetting–drying cycles that enhance SOM degradation (Bottner, 1985). Rhizosphere effects are greater on the surfaces of the aggregates since roots preferentially grow around the aggregate surfaces. Roots control the concentrations and fluxes of soil N by absorbing soil water and soluble N compounds (Frensch et al., 1996; Harper, 1995). Nitrogen is deposited in the rhizosphere as NH_4^+, NO_3^-, and root debris. Janzen (1990) and Janzen and Bruinsma (1993)

reported that up to 20% of total plant N could be deposited to the rhizosphere of wheat plants. The amount of N deposited from pea residue was 48% of belowground N and from barley it was 71% of total belowground N at maturity (Jensen, 1996). Released N *in situ* from decomposing plant roots and shoots contribute to stabilizing soil aggregation processes. Dead roots act as a readily decomposable SOM and cause increased oxygen consumption in rhizosphere (Fisher et al., 1989).

Recent studies showed that soil aggregates develop by adding concentric layers of cations, carbon (Horn 1990; Santos et al., 1997; Smucker et al., 1997) and heavy metals (Wilcke and Amelung, 1996). Short term effects of cropping on soil organic matter and associated rhizodeposition can be determined more quickly when concentric layers are removed from soil aggregates. Santos (1998) showed that 6 weeks after planting ryegrass in a greenhouse potted study, exterior layers of soil aggregates contained 20% newly deposited C while interior regions contained only 8% new C_3–C. Therefore, under field cover crop conditions, it is suggested that recently derived cover crop shoot and root nitrogen could be deposited at greater concentrations on the surfaces of soil aggregates compared to interiors. The objective of this study was to identify the contributions of cover crop root and shoot N to different regions within aggregates ranging from 2.0 to 9.5 mm across in the A_p horizon of a Kalamazoo loam soil. This objective was evaluated during a two-year field study at the Kellogg Biological Station (KBS) of Michigan State University located near Kalamazoo, Michigan.

Materials and methods

Experimental design and treatments

A 2-year field experiment (1997–1999) was conducted on 16 plots (6×10 m) on a Kalamazoo loam soil (coarse-loamy, mixed, mesic Typic Hapludalf) at the Long-Term Ecological Research Site (KBS/LTER) of Kellogg Biological Station in southwestern Michigan. There were four treatments: (1) bare soil control (2) bare soil to which rye (*Secale cereale* L.) shoot mulch was applied (shoot) (3) rye with shoots removed and roots remaining *in situ* (root) (4) rye cover crop roots and shoots (roots + shoots) where rye shoots were cut and placed on soil surface. Each treatment was replicated four times in a randomized complete block design.

^{15}N experiment

Two open-ended PVC cylinders, 30 cm in diameter and 60 cm in depth, were hydraulically inserted into the soil surface through the Ap horizon and into the center of the Bt_2 horizon in each of 16 plots for both years. Two cylinders in each plot were positioned 75 cm apart. Approximately 45 rye seeds were planted in two rows, spaced at 18 cm, within each cylinder of the two rye cover crop treatments. Soil surface in each cylinder was covered with plastic sealed around the walls and each rye row by nontoxic clay sealant on May 22, 1998 and May 10, 1999 just before ^{15}N applications. Pine wood shavings were placed on the plastic to absorb ^{15}N labeled spray mist materials preventing soil surface contamination. Cylinders with no cover crops also received the same treatments.

Rye plants were labeled with ^{15}N by foliar applications of solutions containing 6.39 g $(^{15}NH_4)_2SO_4$ containing 99 atom% ^{15}N dissolved in 9 L of distilled water on May 22, 25, 28, 30, 1998. In the second year of the experiment, ^{15}N was applied on May 10, 12 and 15, 1999.

Three or four splits of this solution were applied to prevent run off or leaf damage by toxicity. Each time equal amounts of ^{15}N solution (approximately 125 mL) were applied manually to each rye planted cylinders using graduated misting spray bottles. Plants within the PVC were covered by clothes-baskets securely anchored to the soil to prevent foliar losses during rain and at night. These covers were removed during sunny days.

Following a 2-week absorption and translocation period, the rye plants were spray-killed with Roundup Ultra without ammonium sulphate that (4.5 L ha^{-1}) was mixed with 186 L ha^{-1} water in early June of 1998 and May of 1999. Approximately 1.32 mL diluted Roundup Ultra solution was applied in each cylinder. Before cutting, the pine wood shavings were removed by vacuum and the plastic protective soil cover and clay sealants were removed from soil surfaces within the

cylinders. Above ground plant parts of rye were manually cut at the soil surface, weighed and subsamples were taken for biomass and ^{15}N analyses. Rye shoots were removed or replaced on the soil surfaces inside the PVC cylinders according to their assigned treatments.

Seeds (6–8) of maize (*Zea mays*, L.) were hand planted into each cylinder. Metal screens with 1 cm openings were placed on the top of the soil and secured with nails to prevent residue losses by wind or animal consumption of maize seeds or rye shoots. Each cylinder received 500 ml water from the soil surface. Two days following maize seedling emergence maize plants were thinned to two plants within each PVC cylinder. Thinned plants were placed on the soil surface to retain 100% of ^{15}N within each cylinder.

Soil sampling

Background soil samples (0–5 and 5–15 cm) were taken from each ^{15}N cylinder using a small (2.5 cm in diameter) sharpened PVC pipe before ^{15}N application. After spray killing of rye, approximately 1 cm soil crust was subsampled from each ^{15}N cylinder to determine if any ^{15}N soil contamination had occurred during labeling. There were no significant differences in the soil concentration sampled from labeled and non-labeled cylinders.

Soil samples for aggregate analyses were extracted from 0–5 cm depths from the soil surfaces by periodically pushing sharpened PVC cores (10 cm diameters) into the soil and removing them using a small garden shovel. Soil samples were removed from the cores. Then, they were air-dried and gently broken into pieces manually. Finally, they were sieved into aggregate fractions of 9.5 mm. In addition, samples, which were sampled in July 1999, were sieved into nine size classes: >9.5, 9.5–6.3, 6.3–4.0, 4.0–2.0, 2.0–1.0, 1.0–0.5, 0.5–0.25, 0.25–0.106, and <0.106 mm and aggregates were analyzed for total N, ^{15}N and SOC.

Aggregate erosion

Concentric layers of aggregates were removed from each aggregate by the meso soil aggregate erosion (SAE) chamber technique (Park and Smucker 2004; Smucker, 2004; Smucker et al., 1998, 1999). Briefly, individual aggregates were placed in small stainless steel cylinders with 2.5 cm diameters and 3.0 cm in depth, whose interior walls were knurled rough and base was enclosed by a 352 micron screen. A stainless steel base was fitted over the screen, at the bottom of the SAE chamber, to catch eroded materials. Each SAE chamber was placed in a glass beaker (100 mL) and secured with sponge packing. SAE chambers were placed on rotary platform shaker (Innova, model 2300, New Brunswick Scientific Co. Inc., New Jersey, USA). Energy to erode aggregates was applied by a rotary shaker set at 200–250 rpm for time durations needed to complete the removal of exterior concentric layers from each aggregate (Figure 1). Most aggregates were eroded into three equal regions (g/g) representing their exteriors, transitional and interiors. Aggregates were selected according to their uniform shapes. Priority was given to the most spherical aggregates with no visible roots to minimize errors originating from peeling plant root residues. External layers were removed by peeling for about 6–90 min at 180–250 rpm. Broken aggregates were discarded and replaced. An Excel spreadsheet was used to calculate and to predict removal of 33 ± 1.5% (g/g) of each soil aggregate.

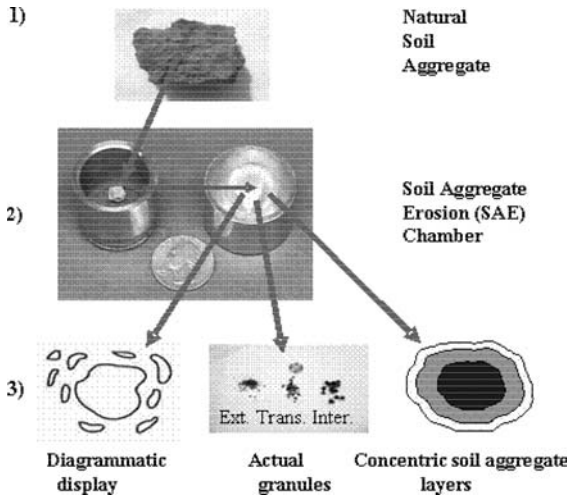

Figure 1. Soil aggregate erosion (SAE) chambers for removing concentric soil layers from air dried soil aggregates ranging from 0.250 to 25 mm across.

Rye root and shoot sampling

Rye root and shoot sub samples were taken before and after the ^{15}N labeling to determine initial and final ^{15}N contents of plant shoots and roots.

Rye root samples were extracted from the top 15 cm depth of soil surface by pressing PVC cores (117 cm^3) into the soil to sample rye roots before and after ^{15}N application. Roots were removed from this sample by developing slurry of distilled water which was poured through a 53 μm screen and the retained roots were washed under water. Fine and white roots and residue remaining on the screen were picked from the sand by tweezers. Rye shoots were washed with distilled water before their application on soil surface. Both roots and shoots subsamples were oven dried at 70 °C for 24 h.

Soil and plant analyses

Following the separation of aggregates into three equal concentric layers, samples were ground in mortar and pestle. Sand was removed by sieving each peeled and ground sample through a 53 μm screen to increase the concentration of the ^{15}N and N in the small sample size associated with each concentric layer of each aggregate. Soil and plant samples were weighed and the total C and N contents were determined by the dry combustion method (Kirsten, 1983) using a C/N/H analyzer NA 1500 series 2 (Carlo Erba Stumentazione, Milano, Italy). Percent ^{15}N in both soil and plant samples were identified by an isotope ratio mass spectrometer, model 2020 (Europa Scientific, Crewe, UK). Calculation of $\delta^{15}N$ and atom $\%^{15}N$ were performed using Equation (1) below (Yoneyama, 1996):

$$\%^{15}N = [(^{15}N/^{14}N_{spl}) - (^{15}N/^{14}N_{std})/(^{15}N/^{14}N_{std})], \times 100 \quad (1)$$

where

^{15}N is the atom % ^{15}N which gives the absolute number of atoms of a N-15 isotope in 100 atoms of total N element.

$$\text{Atom \% } ^{15}N = [^{15}N/(^{15}N + ^{14}N)] \times 100 \quad (2)$$

$$^{14}N = \text{Atom \% } ^{14}N$$

$$\text{Atom \%}^{14}N = [^{14}N/(^{15}N + ^{14}N)] \times 100 \quad (3)$$

spl = sample std = standard (atmospheric N_2)

N derived from labeled residue (concentrations)

$$(\%\text{Ndfr}) = \frac{\text{atom \% 15 N excess}_{\text{soil}}}{\text{atom \% 15N excess}_{\text{labeled rye root or shoot}}} \times 100, \quad (4)$$

where

Atom $\%^{15}N$ excess of soil = (atom ^{15}N of soil in labeled soil) – (atom ^{15N} of soil in unlabelled control)

Atom $\%^{15}N$ excess of rye root and/or shoot = (atom ^{15}N of labeled plants) – (atom ^{15}N of plants before labeling)

Calculations were modified from Stevenson et al. (1998).

Statistical analysis

Treatment effects on measured parameters were estimated by a PROC-GLM procedure using Statistical Analysis System (SAS Institute, 1999). Duncan's multiple range test was used to separate means of measurements. Carbon, nitrogen and ^{15}N contents of exterior and interior layers of soil aggregates were compared by paired *t*-test using Statistical Analysis System (SAS). Correlation analysis was used to determine the relationship between plant and soil parameters. All significant tests were set at the 0.05 level.

Results

Total soil nitrogen (TN)

Soil aggregates, 6.3–9.5 mm across, accumulated the most total N (TN) in their external and transitional layers in July 1999 (Figure 2 and Table 1). Total N ratios of exterior layers to interior regions of 6.3–9.5 mm soil aggregates were 1.06, 1.14, 1.28 and 1.15 for control, shoot, root and root + shoot treatments respectively in July 1999. The greatest increments in total N content in exterior layers were observed in only root treatments. Ratios of external N content to

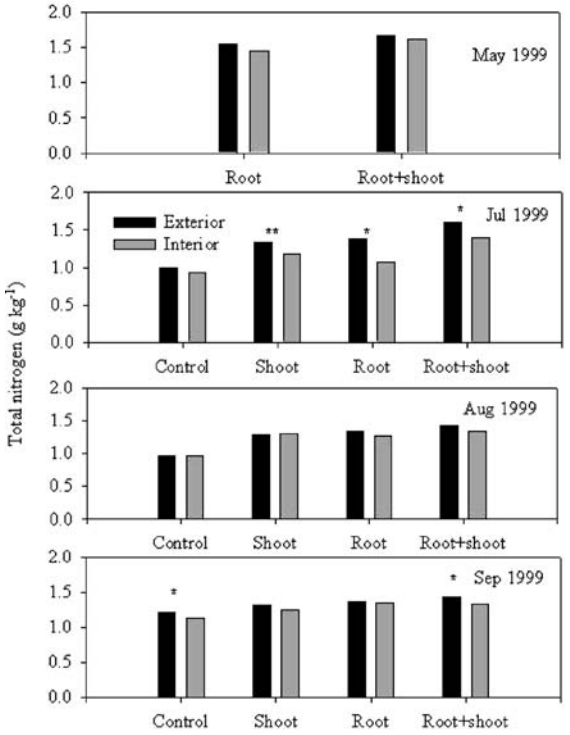

Figure 2. Total nitrogen (TN) concentrations of exterior layers and interior regions of 6.3–9.5 mm soil aggregates from 0–5 cm depth of a Kalamazoo loam soil in 1999. Significant differences between exterior layers and interior regions of aggregates within the same treatment at the $P < 0.05$ (*) and $P < 0.005$ (**) probability levels.

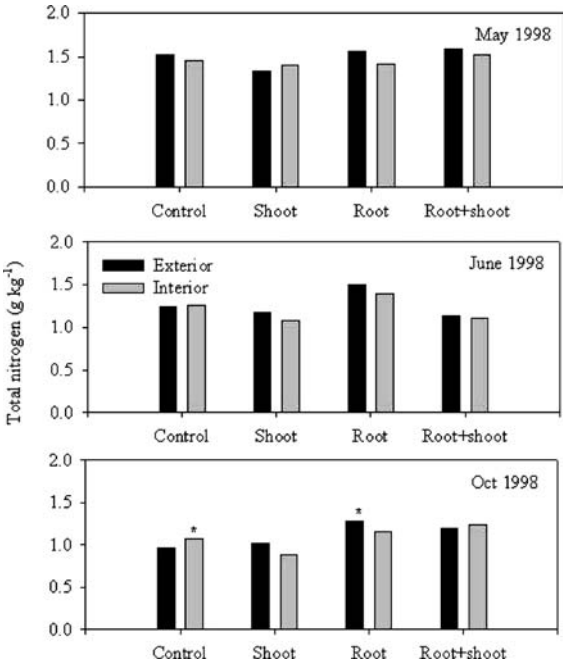

Figure 3. Total nitrogen (TN) concentrations of exterior layers and interior regions of 6.3- to 9.5 mm soil aggregates from 0–5 cm depth of a Kalamazoo loam soil in 1998. Significant differences between exterior layers and interior regions of aggregates within the same treatment at the $P < 0.05$ (*) and $P < 0.005$ (**) probability levels.

Table 1. Total nitrogen and carbon concentrations and C:N ratios of whole aggregates, exterior layers, transitional layers and interior regions of aggregates between 6.3 and 9.5 mm at 0–5 cm depths of a Kalamazoo loam soil on July 7, 1999

Treatment	N (g kg^{-1})				C (g kg^{-1})				C:N			
	Whole agg.	Ext.	Trans.	Int.	Whole agg.	Ext.	Trans.	Int.	Whole agg.	Ext.	Trans.	Int.
Control	0.80c[a]	0.99b	0.95c	0.93c	12.20b	12.01ab	11.67c	13.03b	14.58a	12.24a	12.28a	14.13a
Shoot	0.90bc	1.34a	1.71b	1.18bc	10.50b	9.89b	15.69b	13.53b	11.98b	7.69a	9.17ba	11.56a
Root	1.10ab	1.38a	1.55bc	1.08b	13.00ab	12.60ab	15.61b	13.75ab	11.59b	9.53a	10.07a	12.87a
Root + shoot	1.30a	1.60a	1.87a	1.39a	18.80a	18.03a	18.18a	16.40a	11.90b	11.30a	9.72a	11.77a

[a]Values followed by the same letter within same column and between treatments are not significantly different at $P > 0.05$ according to Duncan's test.

internal N content were between 0.84 and 1.0 for 4.0–6.3 mm and between 0.92 and 1.18 for 2.0–4.0 mm aggregates.

The ratio of TN was not significantly different between aggregate layers in June 1998 (Figure 3). Greater N concentrations resulted in the highest ratios of external N content (Ne) to internal N content (Ni) in July, which diminished through September of 1999 (Figure 2). These fluctuations in N concentrations on external regions of larger aggregates reflect greater flux rate of N movement through larger soil pores associated with aggregates 6.3–9.5 mm across. Exterior layers and transitional (i.e., one-third of the soil mass between

Table 2. Total nitrogen and carbon concentrations and C:N ratios of whole aggregates, exterior layers, transitional layers and interior regions of aggregates between 4.0 and 6.3 mm at 0–5 cm depths of a Kalamazoo loam soil on July 7, 1999

Treatment	N (g kg^{-1})				C (g kg^{-1})				C:N			
	Whole agg.	Ext.	Trans.	Int.	Whole agg.	Ext.	Trans.	Int.	Whole agg.	Ext.	Trans.	Int.
Control	0.90b[a]	1.30b	1.42b	1.30b	11.20b	10.49b	13.30b	13.54a	12.21a	8.08a	9.30a	11.20a
Shoot	1.20b	1.58ab	1.58ab	1.67ab	14.30b	13.65ab	15.70ab	13.59a	11.56a	8.75a	9.93a	8.23ab
Root	1.20b	1.60ab	1.74a	1.90a	13.70b	14.38ab	15.02ab	14.17a	11.85a	9.08a	8.63a	7.47b
Root + shoot	1.60a	1.80a	1.72a	1.80ab	19.70a	17.49a	16.88a	17.00a	12.10a	9.65a	9.81a	9.45a

[a]Values followed by the same letter within same column and between treatments are not significantly different at $P > 0.05$ according to Duncan's test.

Table 3. Total nitrogen and carbon concentrations and C:N ratios of whole aggregates, exterior layers,transitional layers and interior regions of aggregates between 2.0 and 4.0 mm at 0–5 cm depths of a Kalamazoo loam soil on July 7, 1999

Treatment	N (g kg^{-1})				C (g kg^{-1})				C:N			
	Whole agg.	Ext.	Trans.	Int.	Whole agg.	Ext.	Trans.	Int.	Whole agg.	Ext.	Trans.	Int.
Control	1.00c[a]	1.09a	1.43a	1.10a	11.40b	14.73a	13.99b	15.13a	11.67a	14.48a	9.78a	13.75a
Shoot	1.20b	1.04a	1.59a	0.88a	12.60ab	13.67a	13.59b	13.18a	11.00a	15.21a	8.55a	14.98a
Root	0.90c	1.01a	1.36a	1.06a	9.90b	13.12a	14.75b	15.26a	11.56a	14.56a	10.84a	14.40a
Root + Shoot	1.50a	0.91a	1.56a	0.99a	17.20a	13.63a	16.47a	13.96a	11.60a	14.46a	10.56a	14.10a

[a]Values followed by the same letter within same column and between treatments are not significantly different at $P > 0.05$ according to Duncan's test.

Table 4. ^{15}N concentrations of whole aggregates, exterior layers and interior regions of aggregates between 2.0–4.0 mm, 4.0–6.3 mm and 6.3–9.5 mm at 0–5 cm depths of Kalamazoo loam soil on July 7, 1999

Treatment	Atom %^{15}N								
	2.0–4.0 mm			4.0–6.3 mm			6.3–9.5 mm		
	Whole agg.	Exterior	Interior	Whole agg.	Exterior	Interior	Whole agg.	Exterior	Interior
Control	0.370b	0.370b	0.369b	0.370c	0.368d	0.369a	0.370a	0.368c	0.369b
Shoot	0.377a	0.376ab	0.377a	0.377b	0.376b	0.374a	0.370a	0.375b	0.372ab
Root	0.376a	0.373ab	0.372ab	0.374b	0.370c	0.372a	0.371a	0.374b	0.372ab
Root + shoot	0.380a	0.380a	0.378a	0.378a	0.379a	0.373a	0.376a	0.382a	0.373a

[a]Values followed by the same letter within same column and between treatments are not significantly different at $P > 0.05$ according to Duncan's test.

exterior and interior regions) layers of soil aggregate size fractions of 4.0–6.3 and 6.3–9.5 mm across, retained more soil N than bare (Control) soils when either or both root or/and shoot residues of the rye cover crop were present in July 1999 (Tables 1 and 2). Total N concentrations of whole aggregates sampled from rye root + shoot treatments were the greatest among all treatments for all aggregate sizes in July 1999 (Tables 1–3). Soil C contents of 6.3–9.5 mm and 4.0–6.3 mm whole soil aggregates were greatest in rye root + shoot treatments (Tables 1 and 2).

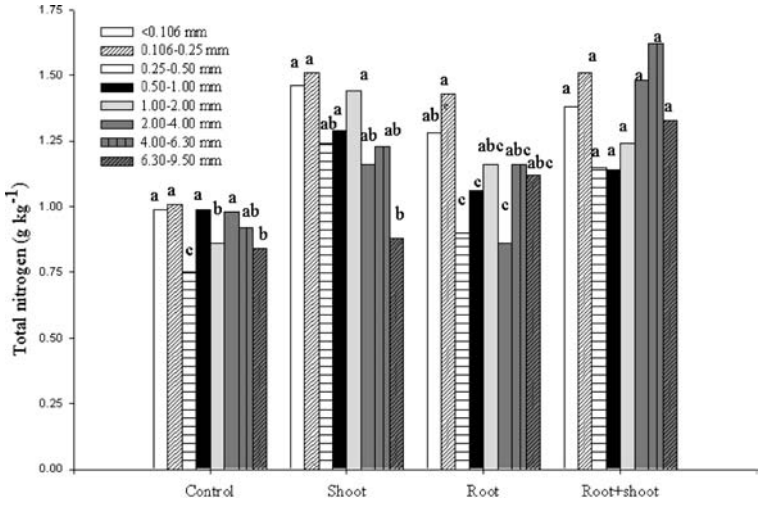

Figure 4. Total soil nitrogen (TN) concentrations in aggregate size fractions sampled from 0–5 cm depths of a Kalamazoo loam soil in July 1999. Values followed by the same letter within each treatment and among aggregate size fractions are not significantly different at $P > 0.05$ according to Duncan's multiple range test, $n = 4$.

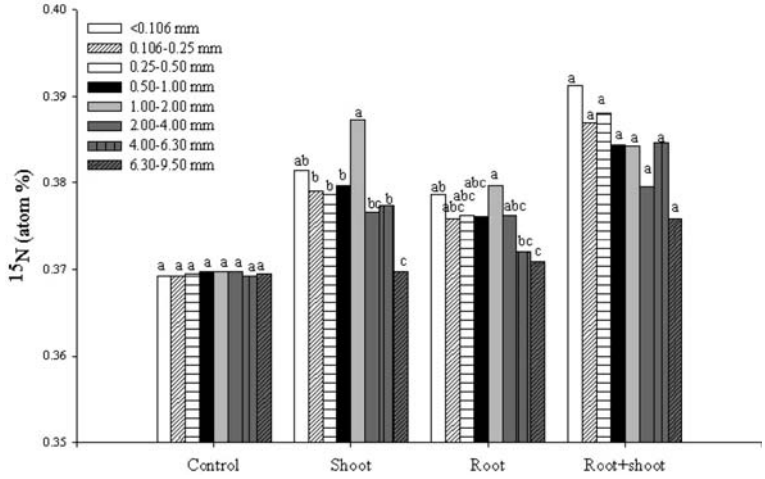

Figure 5. Concentrations of ^{15}N in aggregate size fractions sampled from 0–5 cm depths of a Kalamazoo loam soil in July 1999. Values followed by the same letter within each treatment and among aggregate size fractions are not significantly different at $P > 0.05$ according to Duncan's multiple range test, $n = 4$.

Transition layers of smaller aggregates, 2.0–4.0 mm across, retained significantly higher TN compared to other layers of aggregates (Table 3).

Whole soil aggregates generally had N concentrations that were lower or equal to N in exterior and transitional layers and interior regions of the same size soil aggregates (Tables 1–3). Nitrogen gradients between the external layers and internal regions of soil aggregates of 6.3–9.5 mm across were greatest in July, when compared to August or September of 1999 (Figure 2). Total N concentrations of aggregates decreased from spring to harvest in both years (Figures 2 and 3).

Aggregates smaller than 0.25 mm tended to have more total N in control, root and root + shoot treatments than other sizes. However, statistically they were not significant (Figure 4).

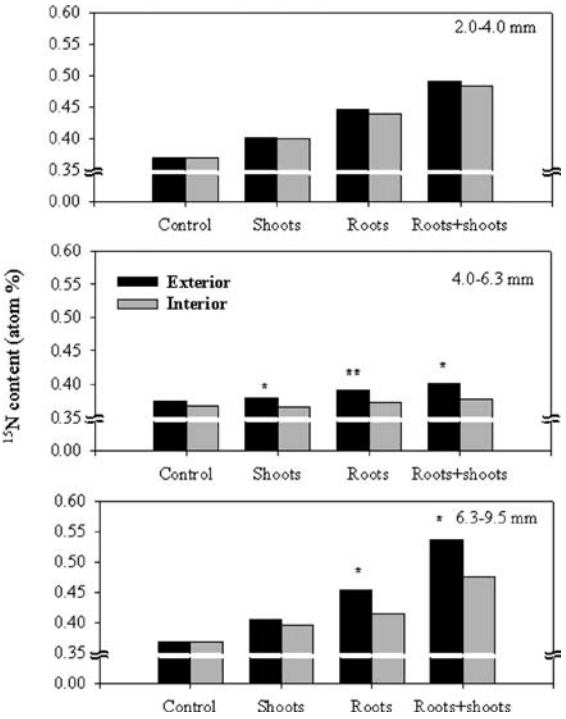

Figure 6. Total ^{15}N contents of exterior layers and interior regions of 2.0–4.0, 4.0–6.3 and 6.3–9.5 mm aggregates from control, shoot, root and root + shoot treatments of the Kalamazoo loam soil on June, 1998. Significant differences between exterior layers and interior regions of aggregates within the same treatment at the $P < 0.05$ (*) and $P < 0.005$ (**) probability levels.

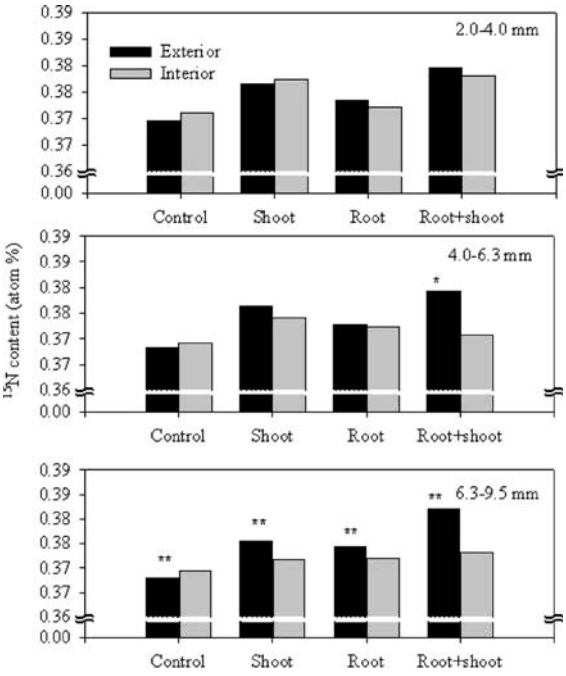

Figure 7. Total ^{15}N contents of exterior layers and interior regions of 2.0–4.0, 4.0–6.3 and 6.3–9.5 mm aggregates from control, shoot, root and root + shoot treatments of the Kalamazoo loam soil on July 1999 . Significant differences between exterior layers and interior regions of aggregates within the same treatment at the $P < 0.05$ (*) and $P < 0.005$ (**) probability levels.

Rye root and shoot derived nitrogen

Distribution of ^{15}N among the full range of aggregate size fractions extracted from bulk soils of different treatments showed trends of greater ^{15}N contents in smaller aggregates and lesser ^{15}N contents in larger aggregates (Figure 5). Microaggregates (< 0.25 mm) retained the second highest rye-derived ^{15}N. This suggests more uniform distributions of ^{15}N within the smaller aggregates and possible ^{15}N gradients established within larger aggregates.

Nitrogen from cover crop roots and shoots could be detected on the exterior layers of soil aggregates of 4.0–6.3 and 6.3–9.5 mm as early as 17 days after rye shoot applications to the soil surface in 1998 (Figure 6). Rye root contributions of N were greater than that of rye shoot N, presumably due to the more rapid decomposition and direct contact of rye roots to soil aggregates. Contrasting gradients of ^{15}N, derived from rye increased with increasing aggregate size (Figure 6). Similar increases of ^{15}N gradients with aggregate sizes were also observed in July 1999, during the second year of these experiments (Figure 7). These results support that most of roots grow preferentially around surfaces of soil aggregates rather than through aggregates. Although concentrations of ^{15}N on surface layers and interior regions of aggregates of 2.0–4.0 mm across were the same as the surface layers of larger aggregates, no gradients of ^{15}N from rye cover crops were observed for aggregates of 2.0–4.0 mm across (Figures 6 and 7). Organic materials derived from rye roots and shoots appeared to be uniformly distributed throughout the aggregates of 2.0–4.0 mm across with minimum ^{15}N gradients at the beginning of the maize growing season (Figures 6 and 7). Contents of ^{15}N within the aggregates of 2.0–4.0 mm across decreased and no gradients were observed at harvest (Figure 8). The ^{15}N gradients developed

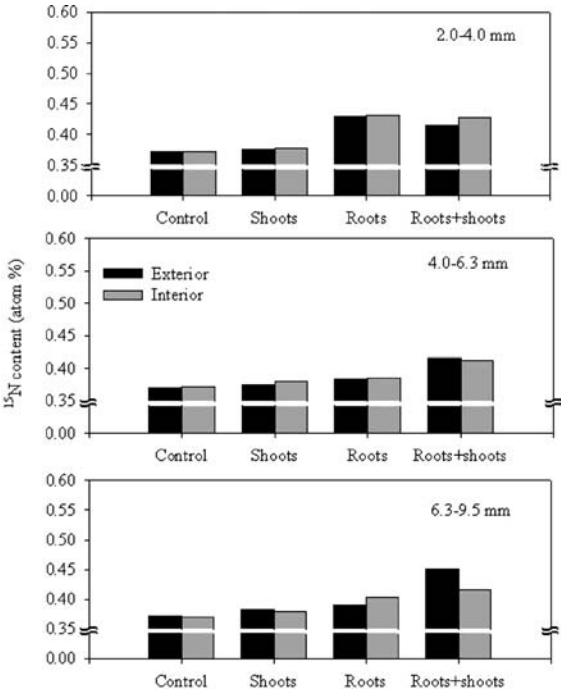

Figure 8. Total ^{15}N contents of exterior layers and interior regions of 2.0–4.0, 4.0–6.3 and 6.3–9.5 mm aggregates from control, shoot, root and root + shoot treatments of the Kalamazoo loam soil on October 1998. Significant differences between exterior layers and interior regions of aggregates within the same treatment at the $P < 0.05$ (*) and $P < 0.005$ (**) probability levels.

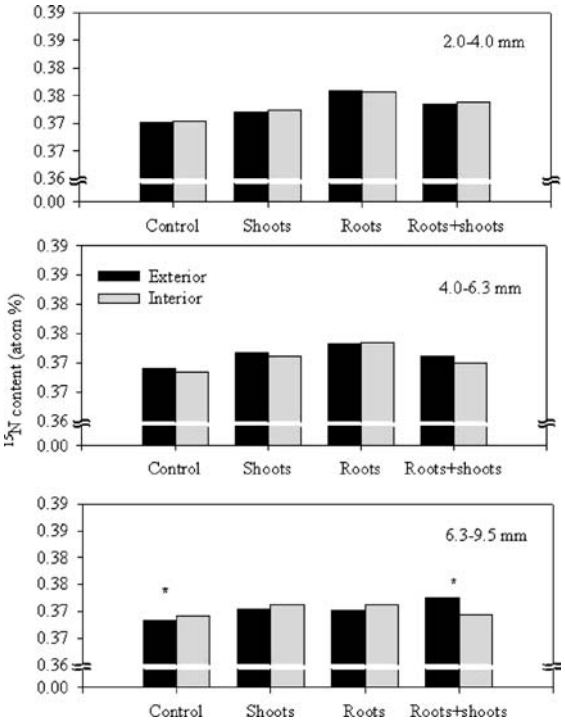

Figure 9. Total ^{15}N contents of exterior layers and interior regions of 2.0–4.0, 4.0-p-6.3 and 6.3–9.5 mm aggregates from control, shoot, root and root + shoot treatments of the Kalamazoo loam soil on September, 1999. Significant differences between exterior layers and interior regions of aggregates within the same treatment at the $P < 0.05$ (*) and $P < 0.005$ (**) probability levels.

within larger soil aggregates, 6.3–9.5 mm across, decreased in October 1998, 116 days after rye shoot application (Figure 8). Nitrogen isotope gradients between external layers and internal regions of soil aggregates of 4.0–6.3 mm across developed early in the summer (Figures 6 and 7) and diminished as the season progressed in both years (Figures 8 and 9). Exterior layer of soil aggregates, 4.0–6.3 mm across, contained similar concentrations of ^{15}N as interior regions at maize harvest (Figures 8 and 9). In summary, there seemed to be a migration of ^{15}N materials from rye roots and shoots into soil aggregates at a constant rate. Early in the season, more ^{15}N migrated into the interior regions of the smallest aggregates, 2.0–4.0 mm across. However, migration of ^{15}N was limited to only surfaces and transitional regions of the larger aggregates, 6.3–9.3 mm across (Figures 6 and 7). Differences of ^{15}N between exterior layer and interior region decreased for the medium sized aggregates, 4.0–6.3 mm across (Figures 6–9).

More rye root-derived N accumulated on the exterior layers of soil aggregates of 6.3–9.5 mm across than rye shoot-derived N (Figure 6). In the first year of experiment, soil aggregate samples were taken 17 days after application of labeled rye shoots on the PVC cylinders. During the labeling period some ^{15}N was transferred from rye shoots to roots and was released to soil by rye roots as root exudates. During applications of Round Up and cutting rye shoots, dead roots continued to release N compounds to the soil. Therefore, more rye root-derived N was deposited on the exterior layers of aggregates in 1998 (Figure 6). In the second year of the experiment, first samples were taken 51 days after application of labeled rye shoots. During that time root derived N was already utilized by maize and shoot derived N concentration was greater than root derived N (Figure 7).

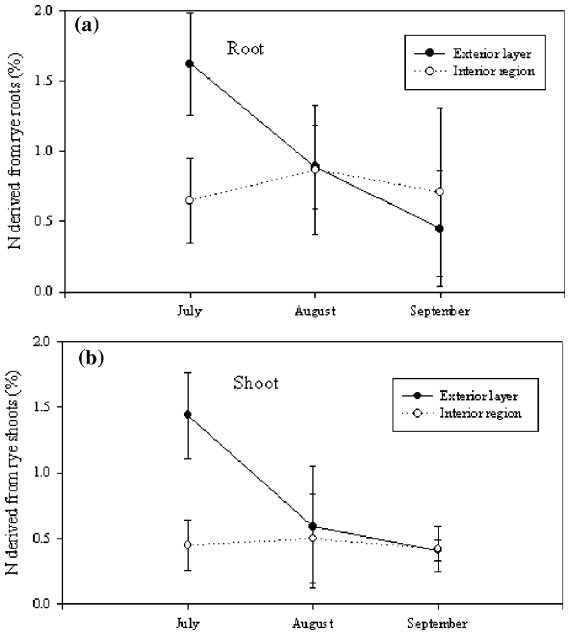

Figure 10. Percentage of N derived (a) from rye roots (%Ndfr) and (b) shoots (%Ndfs) in the exterior layers and interior regions of 6.3–9.5 mm soil aggregates from 0–5 cm depth of a Kalamazoo loam soil in July, August and September 1999. Bars represent standard deviations for $n = 4$.

Nitrogen derived from root (N_{dfr}) and shoot (N_{dfs}) located in the exterior layers diminished from planting to harvest (Figure 10). The percentage of total N derived from rye shoot and rhizodeposition from rye roots was calculated using Equation (2). Exterior layers of aggregates of 6.0–9.5 mm across retained 1.6% of the N_{dfr} in July 1999, three times more than their interior regions (Figure 10). This was slightly greater than the $\%N_{dfs}$. One month later, during the maize growing season $\%N_{dfr}$ and $\%N_{dfs}$ were nearly equal in exterior layers and interior regions of soil aggregates, possibly due to diffusion within larger aggregates and uptake by maize. At harvest, there were greater or equal quantities of cover crop ^{15}N identified within interior regions of the largest soil aggregates, compared to their exterior layers.

Discussion

Greater soil Ne in July reflects greater soil and residue N mineralization which is in agreement with Mendes et al. (1999), who reported that readily mineralizable N content of soil aggregates was greater in June under cereal cover crop treatments than those measured in September. They found significantly less amount of mineralizable N under bare fallow treatments than the cover crop treatments. Transition layers of smaller aggregates, 2.0–4.0 mm across, retained significantly higher TN compared to other layers of aggregates (Table 3) indicating more transient flow of N across the entire regions of these smaller soil aggregates. Smaller aggregates also have better oxygen supply in their internal regions (Sextone et al., 1985). Consequently, the turnover rates of N and possibly SOM appear to be greater in the interior regions of smaller aggregates than larger ones. These reported N gradients suggest alternative formation processes involving concentric deposits of organo-mineral fractions to exteriors of expanding size fractions of soil aggregates.

Whole soil aggregates generally had N concentrations that were lower than or equal to N in exterior and transitional layers and interior regions of the same size soil aggregates. Similar results were observed for some of the treatments by Santos (1998). Total N contents within whole soil aggregates were expected to be in the same range as maximum and minimum N concentrations obtained for exterior layers and interior regions of soil aggregates. One reason for the different (lower) N concentrations for whole aggregates may be the relative heterogeneity of different soil aggregates. To test this hypothesis, we compared 60 soil aggregates having the size fractions between 4.0 and 6.3 mm across and sampled from the 0–5 cm soil depth in the same ^{15}N lysimeter of the cover crop root treatment. These 60 aggregates were analyzed for TN, SOC and ^{15}N. The average TN value was 1.3 g kg^{-1} having a C.V. of 15%. The average SOC value was 12.2 g kg^{-1} with a C.V. of 19% for the same 60 aggregates. Considerable variations at least of these two parameters, among individual soil aggregates from the same volume of soil were observed. Therefore, soil heterogeneity is our best explanation for this discrepancy. Further studies are needed, however, to verify this conclusion. Deposition of ^{15}N, decomposing cover crop roots and shoots, onto soil aggregates caused larger gradients to develop within the concentric layers of larger aggregates during the growth of a

subsequent maize crop. Cover crop root + shoot treatments contributed the most N to soil aggregates than the separate contributions of either *in situ* cover crop roots or shoot mulches.

Decomposing roots and shoots contribute large quantities of C and N to soils (Cheng and Coleman, 1990; Ehrenfeld et al., 1997; Huntjens, 1971; Mary et al., 1993). Although, mineralization of N compounds can be inhibited by living and dying plant components, much of the mineralized N, derived from dead roots or root exudates is immobilized by the microorganisms due to addition of C to the medium. Microorganism utilization of N from the rye cover crop is a highly probable explanation since C:N ratios of rye roots were greater than 50 at the sampling date. If C:N ratio of plant residue is greater than 25:1, N will be taken from the inorganic N pool and decomposition continue slowly until the death of microbial population (Paul and Clark, 1996). Therefore, we have concluded that measured N most probably included recently decomposed plant residue ^{15}N, soil N and microbial biomass N by the sampling date in July 1999. Nitrogen mineralization from rye roots and shoots residues increased in July and resulted in N gradients of 6.3–9.5 mm aggregates (Figure 2). This phenomenon was also reported by Mendes et al. (1999).

Mineralization of SOM may be more rapid on the surfaces of soil aggregates and may be stimulated by growing maize roots (Sanchez et al., 2002). Living roots provide large quantities of C compounds to the surfaces of soil aggregates (Santos, 1998) promoting microbial biomass activities and greater N mineralization (Texier and Billes, 1990). Therefore, maize roots appear to be important C sources for stimulating microorganisms in the soil. Their specific locations on soil aggregates of different sized fractions need further investigation.

When roots preferentially grow on the surfaces of soil aggregates, as discussed above, these roots should increase N mineralization in the external regions of aggregates. Frequent wetting–drying cycles will diffuse more N into interior regions of soil aggregates of all sizes. Mean-free pathways, however, limit the diffusive distance or depths within aggregates of different size fractions. In the presence of roots or high soil water content, highly mobile mineral N located on surface layers of larger aggregates and throughout smaller aggregates can either be absorbed or leached from these respective areas of multiple sized soil aggregates with subsequent diffusion from their interior regions towards their exterior regions.

Direct positive relationships ($r^2 = 0.68$) between ^{15}N ratio of exterior layers to interior regions of soil aggregates (4.0–6.3 mm) and ^{15}N uptake by maize plant support these conclusions. When maize growth continues, concentration of ^{15}N of exterior layers of larger aggregates decreased. In contrast concentrations of ^{15}N in interior region of soil aggregates remained unchanged and ^{15}N in maize plant increased. Most of the ^{15}N presented in the interior regions of soil aggregates greater than 4.0 mm across was preserved at the time of maize harvest.

It was also observed that approximately 20% of the aggregates contained some of the finer roots that had penetrated and passed through soil aggregates. However these soil aggregates containing roots were not selected for analyses. Any fine rye root fragment located within aggregates of any size would result in the deposition of mineralized ^{15}N which could be sequestered within larger aggregates and become unavailable to maize roots unless they penetrated the same larger soil aggregate (Rasse and Smucker, 1998). This is because many of the roots appear to grow around exterior regions of soil aggregates (Allison, 1973, Whiteley and Dexter, 1983).

At harvest, there were greater or equal quantities of rye-N located in interior regions compared to exterior layers of aggregates. Similarly Puget and Drinkwater (2001) reported that after incorporation of vetch cover crop to the soil, amount of root derived carbon decreased from May to October in all aggregate sizes except particulate organic matter of intermediate size aggregates which is preferentially located inside soil aggregates. Our results confirm that most dramatic decrease of both root and shoot derived N is occurred in the exterior layers of soil aggregates between Spring and Fall. In the case of N fertilization, diffusion rate of N from exterior layers to interior regions of aggregates and even leaching could be faster limiting availability of N to the plant. Aggregate sizes used in this study covered only 34% of the total soil by weight. Additional research needs to be conducted on the best management practices for

maintaining more N on surfaces of larger soil aggregates during crop growth as well as sequestering mobile soil N within larger soil aggregates during wet soil periods between cash crops.

Conclusion

This research reports that separating individual soil aggregates into three different layers by SAE chambers provided opportunities to understand short term contribution of cover crop N in soil aggregates and their effect on plant nutrition. Contribution of rye root N was greater than that of rye shoot N to the soil aggregates. Greater root derived N was accumulated on the exterior layer of aggregates. These results supported that roots grow preferentially around the surfaces of soil aggregates rather than intra-aggregates. Organic materials derived from rye roots and shoots were homogeneously distributed across 2.0–4.0 mm aggregates and resulted in minimum ^{15}N gradients.

Results showed that uptake of N is more efficient from the surface of the soil aggregates larger than 4.0 mm. Also, concentric gradients of total N were found in aggregates larger than 4.0 mm. These gradients occurred either during or after formation of soil aggregates. Concentric gradients of rye root and shoot derived N increased with increasing aggregate size and changed with time. The location of N in a soil aggregate is important for plant utilization. There seemed to be a migration of ^{15}N materials from rye roots and shoots into soil aggregates at a constant rate. Early in the season, more ^{15}N migrated to the interior regions of the smallest aggregates, 2.0–4.0 mm across, but was limited to only surfaces and transitional regions of the larger aggregates, 6.3–9.3 mm across. At harvest, more of the ^{15}N located within interior regions of the smallest sized aggregates was withdrawn by maize growth while more ^{15}N remained within the interior regions of the medium sized aggregates, 4.0–6.3 mm across.

All these gradients suggested that the formation and function of soil macro-aggregates were very dynamic processes utilizing many biogeochemical factors. Short term changes in the C, N, and SOM components of soils subjected to agriculture have been difficult to quantify as the methods of measurement have been limited to evaluating composite soil samples that destroy arrangements of aggregates and plant roots in the soil. Therefore investigating soil aggregates especially the location of measured parameters within aggregates should be considered for better soil management systems.

Acknowledgements

This research was supported in part by Corn Marketing Board of Michigan and by the LTER graduate student research grant. Technical assistance of John Ferguson, Greg Parker and Mark Halvorson is gratefully acknowledged.

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Section editor: Y.-G. Zhu