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SOIL RESOURCE HETEROGENEITY AND ECOSYSTEM PROCESSES: EFFECTS OF LITTER AGGREGATION ON SOIL MICROBIAL PROCESSES AND PLANT ROOT FORAGING

By

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ABSTRACT

SOIL RESOURCE HETEROGENEITY AND ECOSYSTEM PROCESSES: EFFECTS OF LITTER AGGREGATION ON SOIL MICROBIAL PROCESSES AND PLANT ROOT FORAGING

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Resource spatial distribution alone can alter ecosystem process rates. Soil resource aggregation within the scale of individual plants can potentially affect primary productivity, plant C allocation, plant N acquisition, decomposition, net N mineralization, N_2O emissions, and ecosystem N retention. To understand how plant litter aggregation affects these processes I distributed *Trifolium pratensis* litter in soil across an aggregation gradient from uniformly distributed to highly aggregated. I examined the effects of this aggregation gradient on decomposition rates and N_2O emissions with two laboratory studies and plant growth and N cycling with two field experiments.

Results show that litter aggregation in soil delayed decomposition for 5 to 7 days and that this delay was likely caused by insufficient O_2 supply to the interior of the litter aggregates. In contrast, aggregated litter stimulated emissions of the greenhouse gas N_2O 7-fold compared to uniformly distributed litter. Elevated N_2O emissions in response to litter aggregation were found regardless if the litter was finely ground or chopped into 5 mm pieces.

Plant root systems can respond to litter aggregation by foraging into resource-rich microsites; however, the degree to which plants benefit from root

foraging into microsites of varying quality is largely unknown. I examined whether root foraging into microsites of varying quality depended on plant growth. I found that *Avena sativa* L. root foraging was positively correlated with growth in response to pairwise choices of contrasting microsite qualities. In contrast, root foraging by *Bromus inermis* L. was not related to plant growth response. In addition, I found that plant N status plays an important role in regulating *Zea mays* L. root foraging under field conditions. These two results suggest that root foraging is only an important mechanism for plant N acquisition under heterogeneous conditions where N is limiting plant productivity.

To better understand the effects of litter aggregation on plant growth and N cycling I distributed ^{15}N-labeled litter across an aggregation gradient and followed the fate of the litter-N into plant and soil N pools. Under N-limited conditions maize was 14% more productive in response to aggregated than uniformly distributed *T. pratensis* litter. In contrast, *Secale cereale* litter aggregation did not affect maize growth. Litter distribution did not affect root to shoot ratio; however, total belowground C allocation appeared to be greater in response to uniformly distributed than to aggregated *T. pratensis* litter. Plant N acquisition was greater in response to aggregated than uniformly distributed litter. Litter aggregation also increased litter-derived N mineralization by 20%, shoot N by 18%, and root N by 33% relative to uniformly distributed litter. I suggest that the spatial coupling of roots and litter aggregates is an important factor regulating C and N cycling in agricultural system with heterogeneous resource distributions and where N is limiting plant productivity.

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Chapter 1: Soil Resource Heterogeneity and Ecosystem Processes

INTRODUCTION

Jensen's Inequality (Jensen 1906) predicts that rates of ecosystem processes such as decomposition will differ between systems with uniform versus heterogeneous distributions of factors that control ecosystem processes if the functional response to those factors is nonlinear. This means that in terrestrial ecosystems where nonlinear functional responses to resources are common and soil resource heterogeneity is the rule (Stark 1994), that the average resource availability in soils will not accurately predict rates of ecosystem processes.

For example, in most soils microbial respiration rate is a nonlinear function of % water-filled pore space (WFPS) (Linn and Doran 1984) such that between 10 and 60% WFPS the relative microbial respiration rate exponentially accelerates with increasing moisture (Figure 1.1). Jensen's Inequality can be illustrated by examining the effects of soil-water distribution on soil microbial respiration in two soils each with the same mean WFPS but different distributions of WFPS microsites. If for example, in a homogeneous soil system all of the soil pores are 45% water filled, the soil microbial respiration rate will be 34% of the maximum rate (Figure 1.1). In contrast, in a heterogeneous soil in which half of the soil pores are 40% water-filled and the other half are 50% water-filled, half of the microsites will have a respiration rate of 20% and the other half will have a

respiration rate of 56% of the maximum (Figure 1.1). This results in an average respiration rate of 38% of the maximum for the heterogeneous soil, which is more than 10% higher than in the homogeneous system despite the same mean WFPS of 45% in each system.

In contrast, if the soil microbial respiration rate were a linear function of %WFPS then these homogeneous and heterogeneous systems would be respiring at the same rate. Exponentially decelerating functional responses to resource availability are also common in soils. For example, denitrification rates display Michaelis-Menten kinetics as a function of soil NO_3^- concentration in the absence of O_2 (Tiedje 1988). In this case spatial or temporal heterogeneity in soil NO_3^- concentration will decrease the mean denitrification rate relative to a uniform NO_3^- concentration. In general, the direction and magnitude of the differences between hetero- and homogeneous systems will depend on the shape of the functional response to the most limiting resource or environmental control and the spatial or temporal variability of that controlling factor.

Field observations, manipulations, and modeling have all contributed to our understanding of the causes and consequences of heterogeneity at multiple spatial scales. Research linking species distributions and process rates to landscape and larger-scale spatial heterogeneity has been a major theme in ecology since the discipline's inception (Wiens 2000). Recent statistical advances (e.g., the introduction of geostatistical analysis to soil science and ecology) (Trangmar et al. 1985, Robertson 1987) as well as advances in microbial ecology and instrumentation have allowed similar questions to be

asked at much smaller scales (Ettema and Wardle 2002). For example, Grundmann et al. (2001) were able to link the spatial distributions of ammonium oxidizing bacteria at < 1 mm scale to the spatial heterogeneity of the oxidation of NH_4^+ to NO_2^- and NO_2^- to NO_3^-. They were further able to model the system to determine that the cause of the community heterogeneity was associated with soil mircoporosity. Several researchers have used micro-electrodes to examine the spatial extent to which hotspots of labile organic matter (e.g., animal manure and decomposing leaves) influence N-mineralization, nitrification, and denitrification (e.g., Nielsen and Revsbech 1998, Meyer et al. 2002). Manipulations of resource heterogeneity have shown that the spatial distribution of plant litter can increase and decrease decomposition, N mineralization, N_2O emissions, and denitrification (Breland 1994, Ambus and Jensen 1997, Magid et al. 2006).

Within the scale of individual plants resource heterogeneity can potentially alter the efficiency of plant nutrient acquisition (see Caldwell 1994). Improvements in nutrient uptake efficiencies under heterogeneous resource conditions relative to uniform conditions appear to be related to both the plant root response to the resource distribution and the mobility and quantity of resources acquired from the microsite. As roots encounter different concentrations of nutrients in soil, ion specific uptake systems are up and down regulated according to the plant's genetics, soil nutrient status, and the plant demand for that specific nutrient (Clarkson 1985). Following the induction of

specific uptake enzyme systems in individual roots, a proliferation of lateral roots is typically observed (Robinson 1996).

For the proliferation response to be beneficial to the plant the resources acquired from a microsite must be of sufficient quantity relative to the specific resource demand of the plant. This root proliferation into heterogeneously distributed nutrient rich microsites is clearly advantageous for the acquisition of relatively immobile nutrients (e.g., phosphorous) that are limited by diffusive flow to the root surface (Kovar and Barber 1989, Caldwell et al. 1991). The nutrient concentration itself appears to be the signal that is inducing the plant root proliferation response (Zhang et al. 2007). If the signal is associated with a large pool of nutrients then the root proliferation response may be a cost effective allocation of root C; however, if the resource is quickly depleted then the proliferation response may be an extravagant expenditure of plant C (Wiesler and Horst 1994, vanVuuren et al. 1996). Hodge et al (1999) found that pasture grasses grown together acquired more N from heterogeneously distributed resources than from uniformly distributed resources only when the source of N was in a complex organic form (e.g., plant litter) and not when N was in the form of amino acids or inorganic N sources. This result supports the idea that under N-limited conditions plants may more effectively acquire N from heterogeneous distributions of the organic N compounds than from a uniform distribution.

Soil tillage in agricultural systems often results in a heterogeneous distribution and layering of crop plant litter in the soil (Staricka et al. 1991). This heterogeneous distribution of crop litter then stimulates maize root growth to

match the litter distribution, although it is unknown if this spatial coupling between roots and litter affects maize productivity (Van Noordwijk et al. 1993). In a cold arid grassland, Jackson and Caldwell (1993) characterized the distributions of many soil properties and found that P was spatially aggregated at scales likely to be influenced by root proliferation of the three main perennial plants in the system. When they added P-enriched microsites to soils between these three perennial plants they found that root proliferation into the microsites was dependent on the plant species present and not necessarily related to the P acquired from the patches (Jackson and Caldwell 1989).

These investigations into soil microbial processes and plant responses to resource heterogeneity have contributed significantly to our understanding of the role of small scale heterogeneity for regulating ecosystem level processes; however, research on soil processes and plant growth responses to heterogeneity have mostly been considered separately. This is despite many longstanding observations that soil and plant processes interact to affect ecosystem level C and N cycling (e.g., Birch 1959). Additionally, many of the factors that cause soil resource heterogeneity (e.g., tillage, burrowing animals, or root distributions) also alter several other aspects of the soil environment, and thus it can be difficult to conclude that resource heterogeneity per se is influencing C and N cycling. This leads to my interest in examining questions regarding the role of soil resource distribution for regulating C and N cycling.

QUESTIONS ADDRESSED

The primary objective of my dissertation is to address the general question of how the spatial distribution of soil microbial and plant resources influences rates of C and N cycling. My general approach for addressing this question is to manipulate the spatial distribution of plant litter across an aggregation gradient in soil and then to follow specific microbial and plant responses separately and then together. In addition, I vary other aspects of the litter or soil environment to mechanistically link the microbial and plant responses to the patterns induced by the litter aggregation gradient.

Plant species vary in their root proliferation response to microsites of inorganic N; however, it is unclear if species vary in their root proliferation response to microsites of N in the form of complex organic matter. Furthermore, it is unknown if plant N demand has the same control over root proliferation into microsites of organic N as into microsites of inorganic N. In the following, I address three questions with the overall objective to examine the soil conditions in which plant root proliferation may play an important role in regulating ecosystem level C and N cycling: 1) does root proliferation vary with microsite quality; 2) does the proliferation response correspond to increased productivity; and 3) how does the plant demand for N influence root proliferation in patches of N-rich organic matter? I address these questions with two experiments: one in the greenhouse and one in the field.

In Chapter 3 I examine the influence of resource heterogeneity on soil CO_2 and N_2O emissions. The emissions of these gases from litter in soils are

controlled by several nonlinear functional microbial responses and thus are likely to differ between uniformly and heterogeneously distributed litter. Additionally, any alteration of C and N cycling in response to litter aggregation will potential affect plant acquisition of litter-N. I examine the influence of resource heterogeneity soil processes by manipulating plant litter across an aggregation gradient. This gradient allows me to address four questions: 1) does the intensity of plant litter aggregation affect litter decomposition and N_2O emissions; 2) does the aggregation effect on decomposition and N_2O fluxes vary with soil moisture and hence diffusional constraints; 3) does plant litter particle size affect CO_2 and N_2O emissions similarly when litter is uniformly distributed and aggregated; and 4) does the presence of growing plants alter the N_2O emissions in response to litter aggregation? I address these questions in two laboratory incubation and one field experiment.

In Chapter 4, I employ two litter aggregation gradients to address the questions: 1) does the aggregation of plant litter influence the growth of individual maize plants in an N-limited system; 2) is the root to shoot ratio and belowground C allocation altered by resource aggregation, and 3) does the distribution of aggregated resources influence maize productivity? I address these questions with two field experiments.

Chapter 5 is a companion to Chapter 4 where I ask how N cycling is influenced by resource aggregation. Specifically, I address three questions: 1) does resource aggregation influence above and belowground plant N acquisition; 2) does resource aggregation influence litter-derived net N mineralization; and 3)

does resource aggregation influence whole system N retention. I address these questions using ^{15}N-labeled plant litter in a field experiment.

Finally in Chapter 6, I summarize the results of the previous four chapters and make linkages between the microbial and plant responses to litter aggregation. Further I make predictions as to under what conditions litter aggregation and soil resource heterogeneity may have significant influences on C and N cycling.

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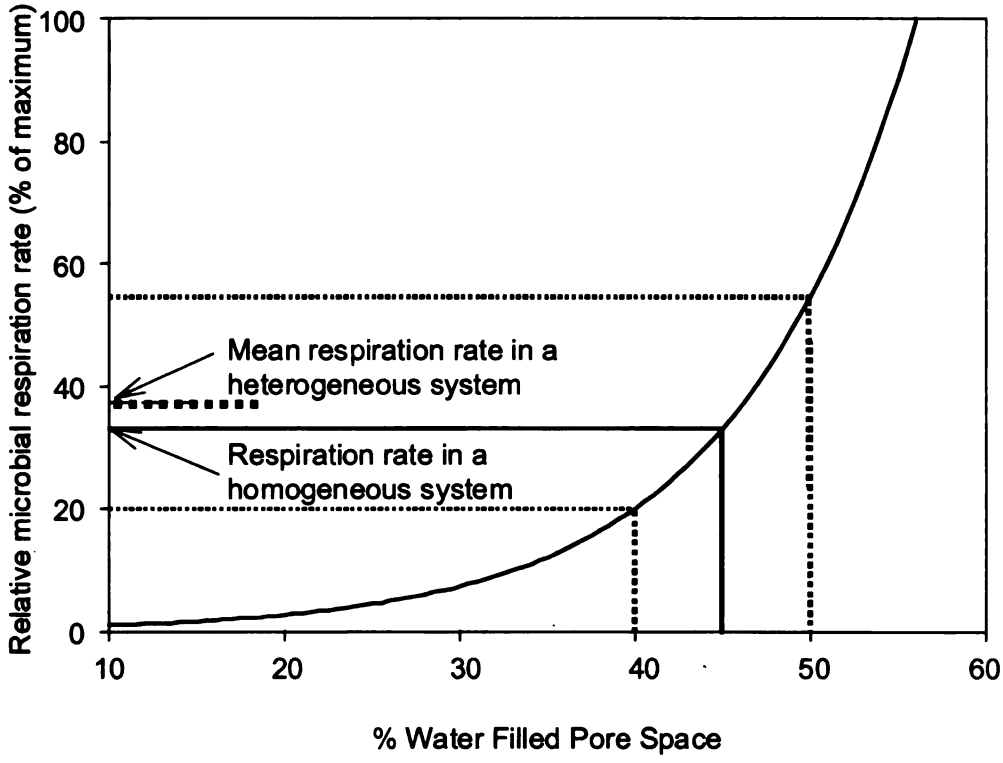

Figure 1.1 Effect of variation in % water filled pore space on mean rates of microbial respiration in homogeneous and heterogeneous environments. The exponential curve represents the microbial respiration functional response to relative soil moisture. The dotted lines represent the respiration rate of heterogeneous system with half of its soil pores are 40% water filled and the other half are 50% water filled. The middle solid line denotes the mean microbial respiration rate in a homogeneous system and the middle dashed line represents the mean respiration rate in a heterogeneous system. This figure is redrawn from Stark, 1994.

Chapter 2. Root Foraging is Responsive to Soil Microsite Resource Quality and Availability

INTRODUCTION

Soil nitrogen (N) availability is the primary constraint on plant productivity in many terrestrial ecosystems, and plant litter-N is often the most important source of soluble N compounds (e.g., NH_4^+, NO_3^-, and amino acids) available for plant uptake. Plant litter and other soil properties are not uniformly distributed in soil and thus plant available N is commonly heterogeneously distributed (Stark 1994). In response, plants have evolved many physiological and morphological adaptations to optimally acquire soil nutrients, including N, from heterogeneous soils (Caldwell 1994). As roots encounter different concentrations of nutrients in soil, ion-pecific uptake enzyme systems are up- and down-regulated according to the plant genetics, soil nutrient status, and the plant demand for that specific nutrient (Clarkson 1985).

Following the induction of specific uptake enzyme systems in individual roots in initially high nutrient microsites, a proliferation of lateral roots is typically observed (Robinson 1996). This selective root proliferation into heterogeneously distributed nutrient rich microsites, also known as root foraging, is clearly advantageous for the acquisition of relative immobile nutrients (e.g., phosphorous) that are limited to diffusive flow to the root surface. In contrast, N uptake efficiency (N acquired per unit of root C expended) can be adversely affected by root proliferation into microsites of NO_3^- if the NO_3^- pool is of insufficient quantity to compensate the plant for the C expended on the

proliferated root biomass. However, in several studies the root proliferation response has been observed after the NO_3^- pool has been depleted (Wiesler and Horst 1994, vanVuuren et al. 1996). Hodge et al (1999) found that pasture grasses grown together acquired more N from heterogeneously distributed resources than uniformly distributed resources only when the source of N was in a complex organic form (e.g., plant litter) and not from amino acids or inorganic N sources. This observation along with the identified genetic basis for NO_3^- induced root proliferation (Zhang et al. 2007) supports the hypothesis that NO_3^- acts as a signal for root proliferation. As a result, if the NO_3^- is associated with a larger pool of plant available N derived from a microsite of N mineralizing organic matter then the proliferation may increase N uptake efficiency; however, if proliferation occurs into a microsite of NO_3^- that is not associated with a source of further plant available N then the proliferation response may be disadvantageous. Thus, examining the influence of the distribution and N mineralizing potential of labile organic matter on root proliferation and plant growth may be necessary to understand the costs and benefits of root proliferation under N-limited conditions.

Wang and Bakken (1997) found that increasing the spatial separation between N-rich and N-poor microsites of plant litter resulted in greater plant N uptake from the N-rich site. By following the soil microbial C and N contents in both the N-rich and N-poor microsites with and without growing plants they were further able to conclude that the roots did not stimulate N mineralization from the N-rich microsite, and rather that the roots intercepted inorganic N from flowing to

the N-poor microsite. This further supports the idea that root proliferation in and around microsites of varying quality has important implications for plant productivity in heterogeneous soil that would not be evident from studying plant-soil interaction under uniform conditions or in microsites of inorganic N alone. It is known that plant species vary in their root proliferation response to microsites of inorganic N (Einsmann et al. 1999, Rajaniemi and Reynolds 2004, Kembel and Cahill 2005); however, it is unclear if species vary in response to microsites of complex organic matter.

Here we present the results of two experiments to address three questions: 1) does root proliferation vary with microsite quality; 2) does the proliferation response correspond to increased productivity; and 3) does the plant demand for N influence root proliferation in patches of N-rich organic matter?

MATERIALS AND METHODS

Experiment #1: Root Proliferation Response to Microsite Quality

We conducted this experiment in a greenhouse at the W.K. Kellogg Biological Station (KBS) in Michigan, USA. Plastic containers (3-L) were laid out in a complete 2x6 Randomized Complete Block Design with 6 replicate containers per block. Factors were 6 microsite quality choices and two plant species (*Avena sativa* L. and *Bromus inermis* L.). The growing media was coarse quartz sand with a bulk density of 1.6 Mg m^{-3} and a 24 h water holding capacity (WHC) of 0.28 g H_2O g $sand^{-1}$. The sand was air-dried prior to initiating the experiment. The microsite qualities consisted of pair-wise combinations of dried and finely ground (A) red clover shoots, (B) red clover roots, (C) green oat

leaves, or (D) oat straw. All possible combinations were used for a total of 6 microsite quality choice factors: red clover shoots vs. red clover roots (AB); red clover shoots vs. oat leaves (AC); red clover shoots vs. oat straw (AD); red clover roots vs. oat leaves (BC); red clover roots vs. oat straw (BD); and oat leaves vs. oat straw (CD).

Each microsite was an intact mass of 0.5 g litter distributed into 12 separate patches per container. To ensure consistent patch distribution we placed a 140 mm diameter acrylic template on top of 1.6 L of sand in each container. The template was 10 mm thick and each quadrant had three 13 mm diameter holes centered 65 mm out from the center of the template. Into each of these 3 holes we placed 0.5 g of plant litter. The same litter type was placed in the 3 holes of the opposite quadrant. In the other six holes we placed one of the other litter types. After filling all 12 holes with litter, the template was carefully removed and an additional 0.5 L of sand was poured into the container to ensure that the microsites stayed in place. Additional sand was added to each container to obtain a uniform mass of 2150 g.

The litter types used in this experiment were chosen because oat and red clover are commonly grown together in low input agricultural systems of the corn belt and the tissues of these two species have a wide C:N range, and well defined chemical characteristics and decomposition dynamics (Berg et al. 1987, Malpassi et al. 2000, Hesselsoe et al. 2001). The C:N for the litters used in this experiment were 12.8, 18.0, 20.2, and 57.3 for the red clover shoots, red clover roots, oat leaves, and oat straw, respectively. Thus the mass of organic N added

to each container varied with the treatment combination: 3 g of red clover shoots contained 97 mg N; of red clover roots contained 59 mg N; of oat leaves contained 61 mg N; and of oat straw contained 23 mg N. The total organic N added to AB, AC, AD, BC, BD, and CD was 156, 157, 119, 119, 82, and 83 mg N container^{-1}, respectively.

A modified Hoagland's nutrient solution less N was used to maintain soil moisture and fertility (Hewitt, 1966). Half strength nutrient solution (800 mL) was added to each container to initiate microbial activity in microsites four days prior to transplanting seedlings. After this initial pulse of solution, the moisture content was adjusted by container every 5 to 7 days to maintain 80% WHC with quarter strength Hoagland's solution.

We chose *Avena sativa* (variety "Ida") as a test plant because of its well known biology as well as its widespread use as a grain crop. *Bromus inermis* was chosen because it has been used in previous studies of root foraging (Rajaniemi and Reynolds 2004) and is a common forage crop. We transplanted one four day old seedling into the center of each container we transplanted to a depth of 30 to 40 mm. The mean initial dry mass of the oat and *B. inermis* seeds were 42.8 mg and 2.7 mg, respectively.

Thirty-nine days after the seedlings were transplanted the plants were harvested. Aboveground shoots were cut at the sand surface and dried at 60°C for 4 d and weighed. We cut the root systems into five sections: the bottom 70 mm of each container was separated from the surface 70 mm; then the surface section was quartered so that each quarter contained 3 of the same litter patches

and the roots that had grown into that quarter. The roots were separated from sand and litter by wet sieving over a1 mm sieve. Approximately 20 to 30 mm of the primary lateral root most proximal to the shoot was separated from the remaining root system and not included in the root length determination. We stored the roots in resealable polyethylene bags with a 10% ethanol solution sufficient to completely submerge the entire root sample. Root length of each quarter was then determined by scanning the roots with a flat-bed scanner (Epson 3180) as the roots floated in an acrylic pan. We analyzed the images for total root length with WinRhizo root image analysis system (Regent Instruments, Quebec, Canada).

We calculated selective root foraging as the proportion of the total root length in the surface 70 mm of sand that was found in the two quarters containing the microsites with greater total N (Campbell et al., 1991). We calculated the mean litter benefit derived from each litter type as the mean aboveground biomass from each treatment containing that particular litter. The degree of choice contrast was calculated by the difference between the mean litter benefit of the two litter choices in a treatment. The choice contrasts were then ranked from least to greatest difference to establish a contrast gradient across the choice treatments.

Experiment #2: Selective Root Foraging across an N Fertility Gradient

We conducted this experiment at KBS on the Long Term Ecological Research (KBS-LTER) site in Michigan, USA (42° 24' N, 85° 24' W). Kalamazoo (fine-loamy, mixed, mesic Typic Hapludalfs) and Oshtemo (coarse-loamy, mixed

mesic Typic Hapluadalfs) soil series co-occur on the site. The soil had been planted to *Medicago sativa* L., *Glycine max* L., and *Triticum aestivum* L. and managed without tillage for the previous 18 years. We laid out the experiment in a Randomized Complete Block Design with three replicates and 6 N application rates plus a no N fertilizer control. Plots were six - 0.76 m wide rows (4.57 m) by 27.4 m in length and were sprinkler irrigated.

On 18 May 2005, maize (Pioneer ® 35Y54) was planted at 71,000 seeds ha^{-1} with a John Deere MaxEmerge. We established the N fertilizer treatments on 1 July 2005 by applying 0, 3.4, 10.1, 13.4, 16.9, 24.6, and 29.1 g N m^{-2} as a urea ammonium nitrate solution with a knife applicator. The maize plants were at the V6 growth stage (Hanway 1963) at this time. Irrigation water was applied from growth stage V10 – R4 to supply 25 mm per week adjusted for precipitation.

In-growth root cores

Root foraging within the N fertility gradient was assessed by comparing maize root growth into litter-amended and unamended control soil cores (Raich et al. 1994). We constructed these in-growth soil cores by pounding a 76 mm diameter x 102 mm length of schedule 30 polyvinylchloride (PVC) pipe with a sharpened edge into the soil and then removing the soil from within the PVC, leaving a ca. 510 mL hole and then these holes were then filled with 630 g of amended or control soil. The soil used in this experiment was collected from a nearby unfertilized plot, sieved (2 mm screen), and allowed to air-dry. The litter amended soil was 20 g litter kg $soil^{-1}$ of red clover shoot litter (same as Experiment #1). We stored both amended and control soils at 4°C for 10 days

prior to field application. The paired (amended and control) cores were located 3.5 m into the plots, within 0.1 m of each other, and in the center of the inter-rows. We deployed the cores in the plots on 22 July 2005 and harvested them on 21 August 2005. The maize plants were at the R1 growth stage when the cores were deployed. This stage is typically thought to be at or just beyond the maximum N uptake stage of the plants (Hanway 1963). We harvested soil from the cores by hammering the sharpened edge of a 64 mm diameter by 100 mm length of PVC pipe into the center of each core. These intact cores were transferred to 1-L polyethylene bags and 0.4 L of 10% ethanol solution was added before storing at 4°C for a maximum of 4 days. The roots were separated from the soil by wet sieving (2 mm), stored in 10% ethanol, and analyzed for total root length by core as described above. The samples were dried at 60°C for 72 hrs and weighed. We harvested maize grain on 14 October 2005 from the center two rows of each plot with a plot-scale combine. Reported grain yields are adjusted to a standard 150 g H_2O kg grain^{-1}.

Statistical Analysis

For the microsite quality choice experiment an analysis of variance (ANOVA) was conducted on each dependent variable using the mixed linear model procedures in SAS v.9.1 (Littell et al. 2005) with block, microsite treatment, and species as independent variables. Multiple comparisons were conducted using a Tukey-Kramer adjustment procedure. Data were checked for homogeneity of variance and normality and were natural log transformed to meet the assumptions of ANOVA as necessary.

The maize grain yield data was fitted to a quadratic plus plateau model (Cerrato and Blackmer 1990) to define plant N saturation using the non-linear regression procedures in SAS v.9.1 (Sita 1994). The source code for this model can be found in Table 2.1.

RESULTS

Experiment #1: Root Foraging Response to Microsite Quality

Aboveground growth of both *B. inermis* and *A. sativa* varied significantly with litter quality treatment (Figure 2.1); however, this response differed between the two species. The average aboveground *B. inermis* biomass was greatest in response to litter treatments containing clover shoots (0.028g plant^{-1}), intermediate in response to oat leaves (0.023g plant^{-1}) and clover roots (0.020g plant^{-1}), and least in response to oat straw (0.008g plant^{-1}). *Avena sativa* responded to litter quality choices in the same rank order as *B. inermis* with an average of 0.24 g plant^{-1} in response to treatments containing clover shoots, 0.17g plant^{-1} in response to oat leaves, 0.16g plant^{-1} in response to clover roots, and 0.10g plant^{-1} in response to oat straw.

The total root length of *A. sativa* in the surface 70 mm of soil varied significantly in response to litter quality treatments, whereas the root length of *B. inermis* did not (Figure 2.2). Total root length of *A. sativa* followed the same rank order response to the litter treatments as the aboveground biomass. The total root length was the greatest in response to litter treatments containing clover shoots (8.99 m plant^{-1}), intermediate in response to oat leaves (7.72 m plant^{-1})

and clover roots (7.50 m plant^{-1}) and least in response to treatments containing oat straw (4.57 m plant^{-1}).

To understand how patch quality influence the distribution of roots within soil, we arranged the choice treatments along a quality contrast gradient. This gradient was determined from a post-hoc evaluation of the litter treatment rank order effects on the aboveground biomass. Thus a quality choice of clover roots and oat leaves represents the weakest choice contrast, because their benefit to the aboveground plant growth was most similar. Whereas the strongest choice contrast was between clover shoots and oat straw is contrast (Figure 2.3). The proportion of total root length of each plant that was located in the two quadrants of the higher quality litter as a response variable indicates selective foraging.

No root foraging differences were detected with *B. inermis* in response to litter quality contrasts (Figure 2.3); however, *B. inermis* produced more root length in patches of oat leaves than clover roots (average selectivity was greater than the 95% confidence interval)(Figure 2.3). In contrast, *A. sativa* demonstrated selective root foraging in all of the litter quality contrasts except the weakest contrast (Figure 2.3). For *A. sativa*, the rank order of the effect size of the foraging selectivity generally followed that of the aboveground productivity ranking. For example, clover roots and oat leaves sponsored similar aboveground productivity and the roots did not distinguish between the two. In contrast, the oat straw and clover shoots produced very different resource levels and the roots appeared to respond accordingly. No differences in *A. sativa* root selectivity were detected between the four intermediate litter contrast treatments;

however, each of these demonstrated selective foraging. For *A. sativa*, the strongest roots selectivity appears to be against oat straw.

Experiment #2: Selective Root Foraging across an N Fertility Gradient

Plot level maize seed production across the N fertilization gradient increased from 990 ± 30 g m^{-2} with no N applied to 1320 g m^{-2} at 11.6 g N m^{-2} of fertilizer according to the fitted quadratic plateau model (Figure 2.4). Mean root length found in the in-growth root cores over the 21 d deployment period decreased from a maximum of ca. 26,800 m m^{-2} with no N added to 800 m m^{-2} at N application levels of 17 to 29 g N m^{-2} (Figure 2.5). The specific root length (SRL) in the in-growth root cores ranged from 0.3 to 12.7 m g^{-1} and averaged 5.3 m g^{-1} across all of the cores (Figure 2.6). The SRL of the plot receiving the lowest two N application rates was fairly constant, averaging 3.3 m g^{-1} with a coefficient of variation (CV) of 16%. At N rates of 10 g N m^{-2} and above, the maize SRL was more variable averaging 5.9 m g^{-1} with a CV of 48%. No differences in SRL were detected across the N gradient between the amended and control in-growth cores.

The proportion of total in-growth core root length found in the amended core serves as an index of selective root foraging. Maize roots were more consistently selective for the amended core when grain yields were limited by N than when maize plants were N saturated (Figure 2.7). Variability in root foraging increased with increasing maize yields. This increase in variability is likely the result of the large differences that can occur between samples of relatively rare occurrences (Hutchings et al. 2000), i.e. when the spatial distribution of a

population or processes is limited to localized patches. The difference between the root length in the amended and the control cores is an alternative representation of selective root foraging (Figure 2.8). The same quadratic plateau regression model that was used to model the grain yield was also fit to the root selectivity-difference data across the N fertilization gradient. The fitted model indicated that the differences in root length between the amended and controls cores were negligible after approximately 15.8 g N m^{-2}.

DISCUSSION

Experiment #1: Root Foraging Response to Microsite Quality

This experiment demonstrates that plant root foraging for microsites of varying quality can be related to the resources acquired and that selective root foraging varies with plant species. Both plant species (*A. sativa* and *B. inermis*) responded to the variation in microsite quality with a similar pattern in aboveground biomass (Figure 2.1). This indicates that each species was likely N-limited, because all of the other nutrient requirements should have been met with the nutrient solution. This is an interesting result given that the range in aboveground biomass between the two species did not overlap. One possible explanation deals with the synchrony of plant N uptake and N mineralization from the microsites. The demand for N by *B. inermis* should have lagged behind that of *A. sativa* due to differences in absolute growth rate, thus N mineralizing from the N-rich microsites (red clover shoots and oat leaves) had a greater chance to flow into the N-poorer microsites where it may have been immobilized for the duration of the experiment. This would have reduced the total available N for *B.*

inermis more than for *A. sativa*. Wang and Bakken (1997) demonstrated that plant N demand can alter N immobilization into N-poor microsites.

The differences in root foraging between *A. sativa* and *B. inermis* (Figure 2.3) may be caused by several factors related to species traits and experimental conditions. Again N availability relative to plant N demand may partially explain these differences. The N-rich microsites may have been mineralizing inorganic N before the *B. inermis* roots could have perceived the patchiness of the inorganic N. This may have allowed inorganic N from the N-rich microsites to have dispersed more widely in the soil, thus *B. inermis* may have experienced a more uniform distribution of inorganic N than did *A. sativa*. Several studies have demonstrated species of different absolute and relative growth rates differ in root foraging traits (Campbell and Grime 1989, Hutchings and de Kroon 1994, Fransen et al. 1999, Aanderud et al. 2003). Further work on nutrient dispersion rates would need to be conducted to understand if these studies may also suffer from patch dispersion prior to the plant nutrient demand. Fitter et al. (2000) point out that patch dynamics maybe as important as plant foraging traits; however, patch dynamics are less intensely researched.

Alternatively, root foraging traits of *A. sativa* and *B. inermis* may differ because of life histories (annual versus perennial, respectively), rooting architecture (Bell and Lechowicz 1994), dependence on soil microbial associates (Hodge 2003), and root turnover rates (Gross et al. 1993, Aanderud et al. 2003). *Avena sativa* has not been studied in root foraging trials to our knowledge. *Bromus inermis* has been used in several root foraging experiments (Rajaniemi

and Reynolds 2004) and has been generally described as a moderately precise forager (Kembel and Cahill 2005); however, our data suggest that *B. inermis*' foraging precision may be context dependent (Figure 2.3). For example, *B. inermis* was the most precise root forager of eight old-field plant species in response to a slow release synthetic fertilizer (Rajaniemi and Reynolds 2004), displayed an intermediate level of foraging compared to three other old-field species in response to a pulse of inorganic nutrient solution (Gross et al. 1993), and did not demonstrate root foraging in response to a choice of plant litter patches in this experiment (Figure 2.3). The nutrient patches used in each of these experiments (Gross et al. 1993, Rajaniemi and Reynolds 2004)(Figure 2.3) differed in form (inorganic versus organic), concentration, and intend duration (nutrient solution, slow release nutrient formulation, and organic matter quality, respectively). Thus it is difficult to speculate if these differences may be due to patch characteristics, genetic variance in foraging traits, or some genetics by environmental interaction.

Experiment #2: Selective Root Foraging across an N Fertility Gradient

The co-occurrence of a grain yield plateau and discontinuation of the selective root foraging suggests that selective root foraging was regulated by the overall N limitation of the plants. In plots limited by soil N availability roots proliferated an average 81% of their total core root length into the amended cores (Figure 2.6). In plots that were not N limited, maize roots did not demonstrate selectively foraging and root growth into the cores was only 3% of the most N-limited plot.

A criticism of the in-growth core method used in this experiment is that it severs intact roots in the portion of the soil where the cores are deployed. Actively growing roots typically respond to the removal of their apical meristems by increasing branching just behind the excision site (Hutchings and de Kroon 1994). Thus the magnitude of the root proliferation into our in-growth cores may be an overestimation of root growth into patches of nutrient rich microsites; however, the relative degree of selective root foraging should be independent of the method.

This study was conducted during the reproductive growth phases of R2-R4 (Hanway 1963). Under non N-limiting soil conditions maize is thought to reduce N uptake rates following pollen shed (R1) and shift to mostly translocating N from nonreproductive tissues to meet the developing grain's N demand (Hanway 1962). In N-limited conditions, however, plants likely continue N uptake into the reproductive phase to meet this demand. Our data suggest that root proliferation into N-rich microsites may be an important component of reproductive phase N uptake if plant available N is limited and heterogeneous distribution within the scale of an individual plant's root system.

The source of plant available N varies with cropping systems, for instance low input system rely more on organic forms of N (legume cover crops and animal manure) to meet plant requirements than conventional cropping systems. The data presented here and elsewhere (Wang and Bakken 1997, Hodge 2006) suggest that root foraging is likely to occur whenever plants are grown in N limited patchy soils; however, this root foraging response may be more beneficial

to plant N acquisition when the source of N is in a complex organic form (Hodge et al. 1999). Drinkwater et al (1998) hypothesized that the coupling of C and N cycling is an important component of the improved N retention they observed in their organic versus conventional cropping systems. Could the spatial coupling of plant roots and N mineralizing plant litter and animal manure be an important component of this improved N retention? In heterogeneous soils, this spatial coupling could potentially increase the flux of N from N-rich microsites to plant roots without increasing the bulk soil inorganic N content (Wang and Bakken 1997), resulting in a decreased potential for this inorganic N to be lost to denitrification or hydrologic leaching. The hypothesis that roots stimulate N mineralization from patches of plant litter does not seem to be important (Bonkowski et al. 2000) despite the rhizosphere having dramatic effects on N mineralization of homogenized soil (Herman et al. 2006). Thus the spatial coupling may be an important component of N conservation in low input organic based cropping systems, but not likely in conventional mineral fertilizer based systems.

CONCLUSIONS

The potential for roots to associate with microsites of varying organic matter quality appears to be dependent on both microsite resource availability and the overall resource limitation of the entire soil-plant system. Our results suggest that root proliferation into microsites is likely only an important plant adaptation when the plant nutrient that is most limiting productivity is heterogeneously distributed.

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Table 2.1 SAS code for fitting a quadratic plateau model to the difference in root mass between the amended and the control cores.

```
dm'output;clear;log;clear;';
OPTIONS NODATE FORMDLIM='_'   LS=75 ; TITLE;
options noovp;
data Nroot;
input trt plot Ngpm Ggpm Amass Cmass Alength Clength Asrl Csrl
Agpm  Cgpm Ampm Cmpm  Mgpm Mmpm Dgpm  Dmpm Prtlen Protms;
y = dmpm; *dmpm = difference in root mass between A and C
cores;
x = ngpm; *ngpm = N rate, g N m-2;
*A= amended and C= control cores, gpm = g m-2, length = cm
root per core, srl = g m-1 of root, D = difference between A
and C, G = grain yield;
cards;
run;
proc sort data=Nroot;
by Ngpm;
run; quit;
title 'Quadratic Model with Plateau';
   proc nlin data=Nroot; /*nonlinear regression procedure in
SAS*/
      parms a=3000 b=0.08 c=-0.0001;

      x0=-.5*b / c;                  * Estimate join point;
      if x<x0 then                   * Quadratic part of Model;
         model y=a+b*x+c*x*x;
      else                           * Plateau part of Model;
         model y=a+b*x0+c*x0*x0;

      if _obs_=1 and _iter_ =.  then do;
         plateau=a+b*x0+c*x0*x0;
         put /  x0= plateau=  ;
         end;
      output out=yhatdmpm predicted=yp u95m=u95i l95m=l95i ;
   run;
     /* Setup for creating the graph */
   legend1 frame cframe=ligr label=none cborder=black
          position=center value=(justify=center);
   axis1 label=(angle=90 rotate=0) minor=none;
   axis2 minor=none;
   proc gplot; *plots the model output and the raw data;
      plot y*x yp*x /frame cframe=ligr legend=legend1
      vaxis=axis1 haxis=axis2 overlay ;
   run;
quit;
proc print data=yhatdmpm;
run; quit;
```

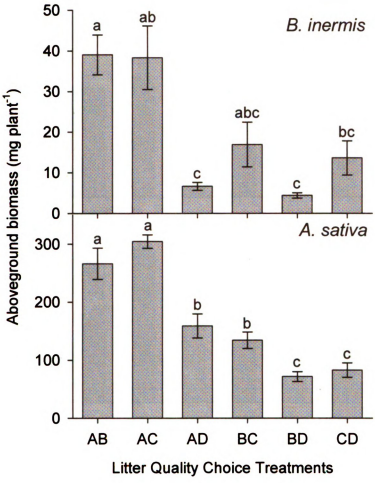

Figure 2.1. Mean aboveground biomass of *B. inermis* (top) and *A. sativa* (bottom) in response to litter choice of treatments: AB = clover shoots/clover roots; AC = clover shoots/oat leaves; AD = clover shoots/oat straw; BC = clover roots/oat leaves; BD = clover roots/oat straw; CD = oat leaves/oat straw. Different litter above the bars indicate significant differences (P = 0.05).

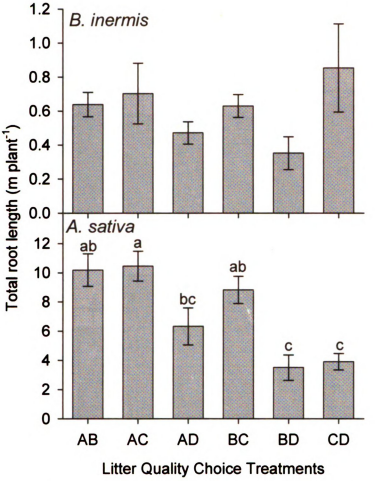

Figure 2.2. Total root length in the surface 70 mm of sand in response to litter quality choice treatments (same as Figure 1). Vertical bars represent treatment means +/- one standard error. Different letters above each bar indicate significant differences (alpha = 0.05).

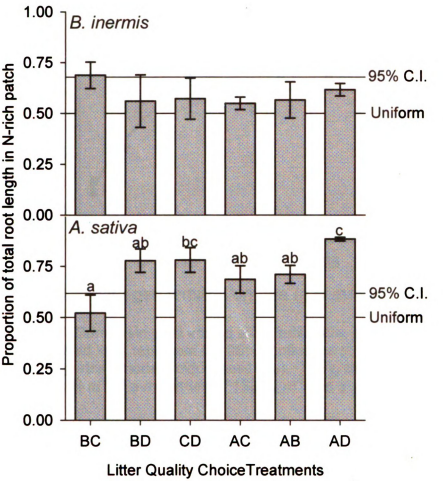

Figure 2.3. Proportion of root length growth of *B. inermis* (top) and *A. sativa* (bottom) into patches of higher quality litter. Uniform line at 0.5 represents an even distribution of roots into quadrants of each litter type. The 95% C.I. line represents the upper confidence interval around the uniform distribution line. Treatment means falling on or below the 95% C.I. are statistically indistinguishable from the uniform root length distribution, i.e. no selective foraging. Different letters above the vertical bars indicate significant differences (Tukey-Kramer adjustment for multiple comparisons).

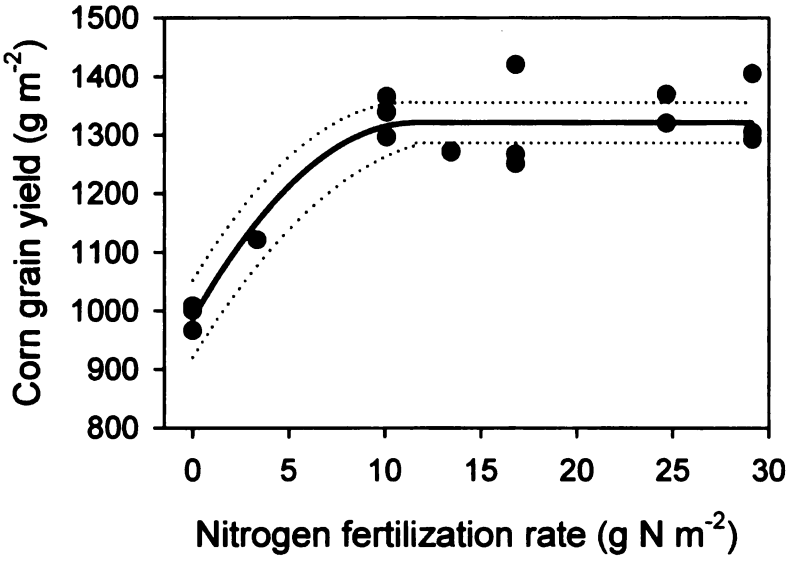

Figure 2.4. Corn grain yield across an N fertilization gradient. The top and bottom dotted lines represent the 95% confidence intervals around the fitted quadratic plateau model (middle solid line). The grain plateau was reached with 11.6g N m^{-2} at a maximum of 1321 g m^{-2} and a minimum of 986 g m^{-2}.

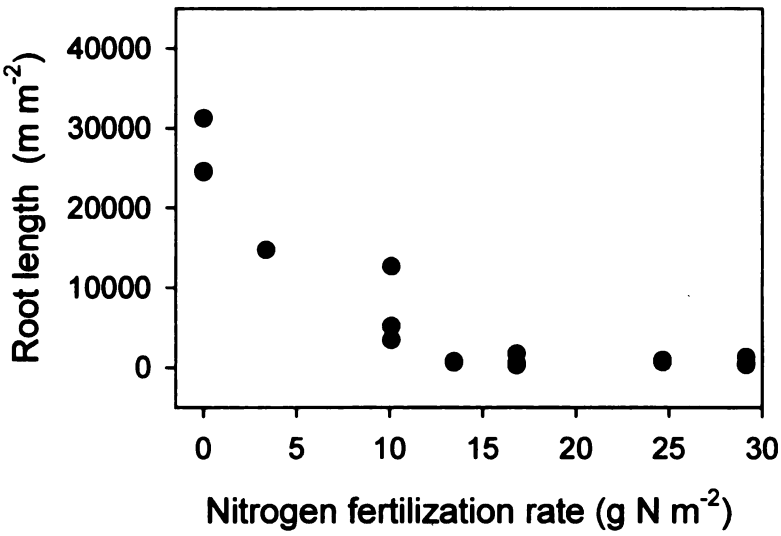

Figure 2.5. Mean corn root length in in-growth cores across an N fertilization gradient.

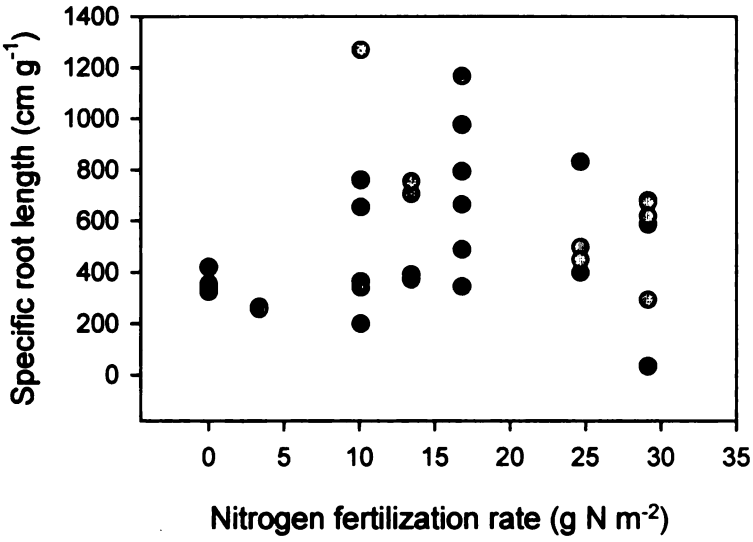

Figure 2.6. Specific root length of roots in amended and control in-growth cores across our N fertilization gradient. The gray circles indicate the litter-amended cores and the black circles indicate the unamended control cores.

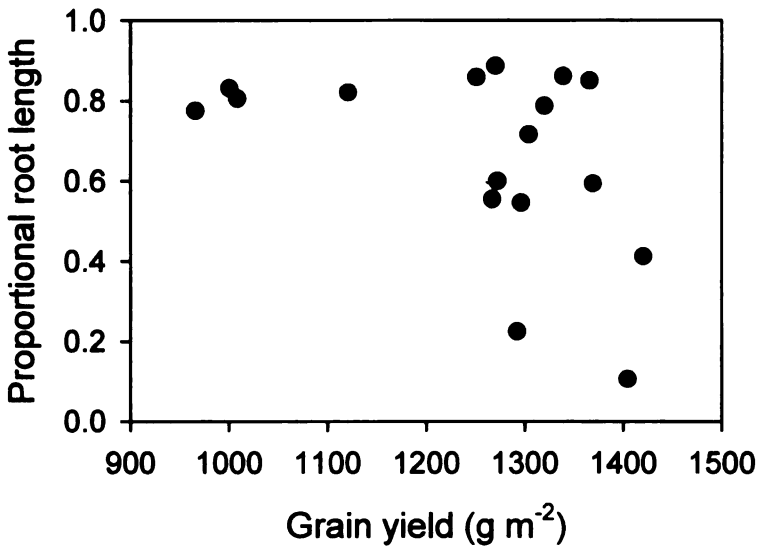

Figure 2.7. Proportion of total in-growth core root length found in amended cores as a function of the maize grain yield.

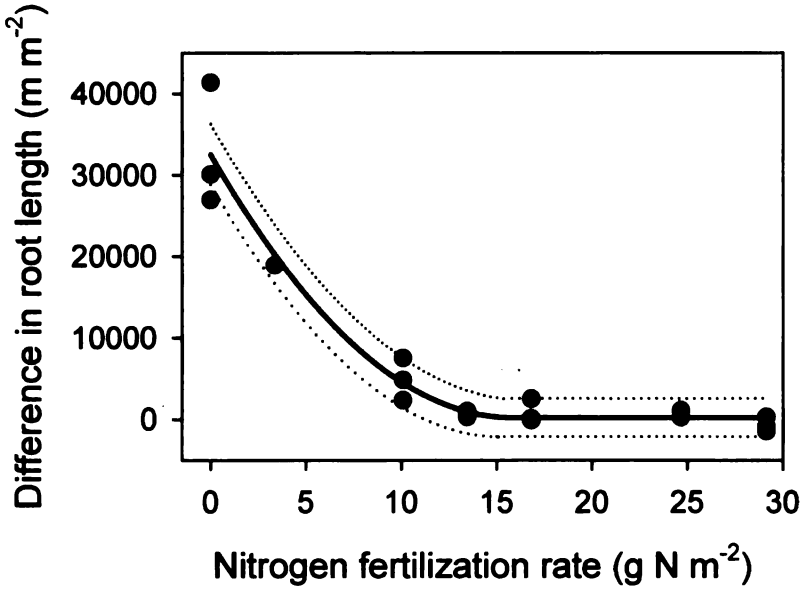

Figure 2.8. Selective maize root proliferation into amended and unamended root in-growth cores across our N fertilization gradient as indicated by the difference in root length between amended and unamended cores (root length in amended core minus length in unamended core). The top dotted line depicts the upper 95% confidence interval, middle line solid depicts the fitted quadratic plateau model and the lower dotted line indicates the lower 95% confidence interval. The maximum difference occurred at 32,500 m m^{-2} +/- 1765 S.E. and the plateau of 226 m m^{-2} was reached at 15.8 g N m^{-2}.

Chapter 3. Litter Aggregation has a disproportionate influence on Nitrous Oxide Flux

INTRODUCTION

Biogenic greenhouse gas emissions from soils are typically studied under in-situ conditions or in homogenized laboratory incubations. The former requires intensive monitoring to obtain realistic estimates of emission rates, whereas the latter is intended to identify the cellular-level controls on these processes. Extension of mean in-situ soil conditions to process rates using predictive models based on the controls identified under homogeneous conditions has proven insufficient. This is in part because soil properties and process rates are temporally and spatially heterogeneous at multiple scales (Groffman and Robertson, 2007).

Jensen's Inequality is the fundamental basis for the prediction that process rates will differ between systems with uniform and heterogeneous distributions of process controls if the functional response to that control is nonlinear (Jensen 1906). The soil microbial processes responsible for soil surface CO_2 and N_2O fluxes, namely decomposition and denitrification, have nonlinear responses to the availability of many resources (e.g., soil moisture, Linn and Doran 1984). If spatial or temporal resource distribution at microbial scales has significant effects on biogeochemical processing rates then our understanding of larger scale processes may be challenged.

The incorporation of plant litter into soil is a potentially important source of microbe-scale resource heterogeneity in many terrestrial ecosystems; however,

most studies of litter decomposition and litter's influence on N_2O fluxes have uniformly distributed the litter in soil (Magid et al. 2006). The few exceptions to this have compared the influence litter distribution, either distributed uniformly or placed in a horizontal layer, on decomposition and N cycling processes (N mineralization, N_2O emissions, denitrification, and leaching).

The results from these experiments are mixed. For example, Breland (1994) compared the decomposition of red clover (*Trifolium pretense L.*) shoots when uniformly distributed versus when placed in a single horizontal layer in a loamy soil. The layered litter initially decomposed more rapidly than the uniformly distributed. Breland postulated that the difference in litter and soil physical contact between the distributions provided for different levels of physical protection from microbial attack. He also suggested that this physical protection was responsible for the reduced denitrification rates observed in the uniform litter distribution. In contrast, Ambus et al. (2001) found that uniformly distributed wheat (*Triticum aestivum L.)* and alfalfa (*Medicago sativa L.*) litter produced 6.5 and 1.6 times more N_2O, respectively, than layered. By following N mineralization in response to these two spatial distribution, Ambus et al. (2001) hypothesized that the observed differences in N_2O fluxes were due to a greater N limitation in the layered litter.

Magid et al (2006) followed decomposition and soil inorganic N in response to layered and uniformly distributed corn (*Zea mays L.*) stalks, sheep (*Ovis aries L.*) manure, and rapeseed (*Brassica napus L.*) stems. Each material initially decomposed more rapidly when uniformly distributed into a loamy sand

soil; however, this was followed by a sustained period of more rapid decomposition in the layered litter. They also suggest that a temporary N limitation during decomposition of these materials is exacerbated when the materials are layered due to physical constraints on NO_3^- diffusion from the bulk soil.

The spatial co-occurrence of soil micro- and mesofauna with decomposing organic matter (Van Noordwijk et al. 1993) may also explain some of the differences in C and N cycling in uniformly distributed versus layered litter (Griffiths 1994). For example, Bonkowski et al. (2000) found greater population densities of bacterial feeding nematodes and protozoans in soils containing layered fresh *Lolium perenne* leaves than in uniformly distributed leaves. These enhanced microbial grazer population densities corresponded to an increase N mineralization from the litter. The favored hypothesis to explain this response was that the concentrated resource allows for more trophic levels to spatially co-occur, and thus mineralize C and N at a greater rate (Clarholm 1985).

A uniform distribution of litter of varying particle sizes may also behave similar to a gradient in litter aggregation. The larger particle sizes have less soil to litter contact and are numerically fewer than smaller particles of litter. Fine grinding of plant litter has both stimulated (Angers and Recous 1997, Ambus et al. 2001) and inhibited (Breland 1994, Shelp et al. 2000) litter decomposition and N cycling rates. In particular, N_2O emissions have ranged from 50% higher (Ambus et al. 2001) to 20% lower (Shelp et al. 2000) in soils with finely-ground litter versus coarsely chopped litter.

Three main hypotheses have been forwarded to explain how organic matter aggregation may alter soil microbial activity and CO_2 and N_2O emissions: 1) resource density dependent expansion of trophic levels to include soil microfaunal grazers (Clarholm 1985, Bonkowski et al. 2000); 2) release from the soil's physical protection (Breland 1994); and 3) resource diffusional constraints (e.g., NO_3^- and O_2) (Myrold and Tiedje 1985, Magid et al. 2006). Because the second and third hypotheses have only been tested in systems comparing a single litter layer versus a uniform distribution it is difficult to extend these results across gradients in aggregation or environmental conditions.

Our objective is to examine the influence of resource heterogeneity on CO_2 and N_2O emissions by manipulating plant litter across a gradient of aggregation. By varying the intensity of litter aggregation we are able to alter the level of physical protection and diffusional constraints to address four questions: 1) does the intensity of plant litter aggregation affect litter decomposition and N_2O emissions; 2) does the aggregation effect on decomposition and N_2O fluxes vary with soil moisture and hence diffusion constraints; 3) does plant litter particle size affect CO_2 and N_2O emissions similarly when uniformly distributed and aggregated; and 4) does the presence of growing plants alter the N_2O emissions in response to litter aggregation? We address these questions in two laboratory studies and a field experiment.

MATERIALS AND METHODS

Experiment #1: Litter Aggregation and Soil Moisture

We used a 2x4 factorial experiment laid out in a Randomized Complete Block Design (RCBD) with 5 replicates to address the question of how litter aggregation and soil moisture content affect decomposition and N_2O emissions. Soil moisture (50% or 80% water filled pore space - WFPS) and plant litter aggregation (1, 3, or 9 patches or uniform distribution) in the soil were the manipulated factors. We mixed coarse sand 1:1 into a composite soil composed of the surface 0.4 m of soil from the W.K. Kellogg Biological Station Long-Term Ecological Research site (KBS-LTER), including both Kalamazoo (fine-loamy, mixed, mesic Typic Hapludalfs) and Oshtemo (coarse-loamy, mixed mesic Typic Hapluadalfs) series. The soil mixture was air-dried and stored for ca. 18 months prior to use. Immediately prior to initiating the experiment five 10 g subsamples of soil mixture were extracted with 100 mL of 1M KCl each for inorganic N concentration determination (Sollins et al. 1999) and indicated that the mixture contained 21.4 μg NO_3^--N g $soil^{-1}$ and 1.0 μg NH_4^+-N g $soil^{-1}$ (colorimetric determination on a OI Alpkem 3550 Flow analyzer).

We used red clover (*Trifolium pretense*) shoots as the litter. The clover was grown in sand in the greenhouse, fertilized with a modified complete Hoagland's nutrient solution (Hewitt 1966), and harvested before initiation of the reproductive phase. The shoots were cut at the sand surface, dried for 4-5 days at 55°C, coarsely-chopped to pass a 10 mm screen with a Wiley mill and then finely-ground in a Cyclotec® 1093 sample mill to pass a 1 mm screen. Eight subsamples of the finely-ground litter were analyzed for C and N content with a

Costech ECS 4010 CHNSO elemental analyzer and found to contain 413 g C kg litter^{-1} and 32 g N kg litter^{-1} giving it a C:N ratio of 12.9.

Incubation setup

We conducted the litter incubations in 2.6 L (0.15m in height) round polyethylene containers. The container lids were fitted with two 6.4 mm diameter threaded polyvinylchloride reducer couplings: one was attached to a three-way stopcock to allow gas sampling with a 10 mL syringe with, and the other was attached to a 28 gauge hypodermic needle hub to act as a vent. We sealed the lids onto the containers only when gas sampling. In between samplings the containers were covered with 1.0 mil low density polyethylene bags to minimize moisture loss while allowing O_2 and CO_2 gas exchange. We incubated the containers on a bench top out of direct sunlight. Daily minimum and maximum air temperature was recorded with a digital thermometer placed in the center of the containers: temperatures ranged from 21.7 to 23.5°C and averaged 22.7°C throughout the 39 day incubation.

A litter aggregation gradient was constructed in each container by distributing 4.5 g of dry finely ground red clover shoots into one patch (4.5 g of litter per patch), 3 patches (1.5 g of litter per patch) or 9 patches (0.5 g of litter per patch), or uniformly mixed into a band of soil (Figure 3.1). We constructed the patches of litter by first placing 850 mL of soil mix into each container and then a template for the litter placement was pressed into the soil. The template was a 140 mm diameter circular sheet of acrylic with one 21.3 mm, three 14.3 mm, or nine 8.3 mm diameter plastic syringe cylinders, less the needle hub end,

adhered perpendicular to the sheet. The rubber tips of the syringe plungers were removed and the plungers were placed in the syringe cylinders as the template was pressed into the soil to displace consistent volume of soil. The litter was then placed into the syringe cylinders and topped off with soil. We pushed the plungers down into the cylinders as the template was raised so that the litter remained in the soil as individual column shaped patches just below the surface of the soil. The mean depth of the litter patches was similar across the gradient (Figure 3.1). An additional 400 mL of soil was then added on top of the litter and initial soil. The uniform treatment was constructed by first adding 250 mL of soil to the containers, then 600 mL of a litter and soil mixture to which was then added 400 mL of additional soil. We added soil to all of the containers to bring each to 1940 g.

Soil moisture treatments were established by adding 260 mL (50% WFPS equivalent) or 415 mL (80% WFPS equivalent) of water (purified by reverse osmosis) slowly to each container. Containers were weighed weekly and water was added to maintain a constant moisture content.

CO_2 and N_2O flux determinations

Carbon dioxide and N_2O gas fluxes across the soil surface were determined by incubating the containers for 35 to 45 minutes with the lids to allow these gases to accumulate in the headspace above the soil to be sampled (Holland et al. 1999). Gas samples were collected at four times with 10 to 12 minutes between samplings. The headspace was sampled by transferring 20 mL of gas from the container headspace to 5.9 mL glass vials outfitted with rubber

septa using a 10 mL plastic syringe and 22 gauge hypodermic needles. We determined N_2O concentrations on a gas chromatograph (GC) outfitted with an electron capture detector. An infrared gas absorption analyzer (LI-Cor 820) in series with the GC was used to determine the CO_2 gas concentration. We regressed the sample CO_2 and N_2O concentrations with a linear model against sampling time to determine the gas flux rate.

Experiment #2: Litter Aggregation and Particle Size

To assess the effects of litter aggregation and particle size on decomposition and N_2O fluxes we used the same soil, litter, and incubation setup as Experiment #1 in a 2x2 RCBD with four replicates per block for a total of 16 experimental units. The litter distribution treatment consisted of the uniform litter distribution and the 3-patch distribution only, and the particle size treatment was composed of the finely-ground and the coarsely-chopped red clover shoots. Soil moisture was maintained at 50% WFPS throughout the incubation. Gas fluxes were measured from these containers on 9 sampling dates across the 18 day incubation. The air temperature during this incubation ranged from 21.9 to 24.5°C and averaged 23.3°C.

Experiment #3: Litter Aggregation with Growing Plants

Plant roots alter many of the same soil properties that control microbial activity in soils. To assess the influence of litter aggregation on N_2O fluxes in the presence of growing plants in the soil we scaled up the design used in Experiment #1 to accommodate a growing maize plant in 50-L containers that were placed in the field. We will describe the details of this experimental setup

briefly, because they are described elsewhere (Loecke Chapter 5). The experiment was conducted on the KBS-LTER using a similar soil mixture as experiments #1 and #2. We used a RCBD with 4 replicates per block and 5 soil amendment treatments consisting of four litter distributions (8, 24, and 72 patches and uniform) and a control +N fertilizer treatment. Each of the 20 experimental units were composed of a 50-L black plastic container filled with the soil mix, amendment, and a single maize plant (Pioneer ® 35Y54).

Litter application

We distributed finely-ground red clover litter into eight – 4.69g patches, twenty-four – 1.56 g patches, seventy-two – 0.52 g patches or a uniformly distributed 37.5 g patch of litter in the soil; however, because we did not want to disturb the litter patches when planting the maize no patches were placed within the center 150 mm of the container (Figure 3.2). We constructed the litter distribution treatments by: placing a temporary circular template of the same diameter as the inside of the 50-L container on top of 40 L of soil mix; adding the litter to the template; removing the template; and then adding 10 L more soil mix on top of the litter. The patches were distributed at a mean depth of 100 mm below the soil surface and application process lasted three days from May 22-24, 2006 during which no precipitation was allowed into the containers. The template was a similar to that used in experiments #1 and #2. The uniform treatment was constructed by mixing 37.5 g of litter with 4 L of soil taken from to the containers, then adding to the litter-soil mix back into the outer ring of the soil in the container (Figure 3.2).

Planting and fertilizing

The overall design of this experiment is to test N cycling responses to litter distribution in a system where N was the only soil nutrient limiting plant growth. To achieve this we applied 250 mL of nutrient solution containing 0.5 P g, 1.25 K g, 0.25 S-SO_4 g, 0.27 Ca g, and 0.07 Mg g to the soil surface of each container on July 6, 2006. In addition the control +N containers received 1 L of solution containing 1.6 g N as $NaNO_3$ and litter treated containers received 1 L of RO (reverse osmosis) water. Supplemental watering (2 L container^{-1}) was conducted on August 13 and 22.

Soil surface N_2O flux

We used removable static chambers to measure soil surface N_2O flux (Holland et al. 1999). Each chamber lid sealed around the outside of the container and the plant stem (Figure 3.2). The chamber lids were modified 120-L refuse container and lid with a 60 mm wide slit removed from on edge to the center of the lid to accommodate the plant stem. Latex sheeting was secured to the plant stem and chamber lid to complete an air tight seal. Deployment of the chamber lid required ~3 minutes and remained on the container for a maximum of 70 minutes during the sampling. We used a similar sampling and analysis procedure as above.

Statistical Analysis

All flux data were natural log transformed to meet the analysis of variance (ANOVA) assumption of homogeneity of variances. For experiments #1, we used a repeated measure ANOVA on the full factorial design with block,

moisture, litter distribution, and sampling date as independent fixed variables with sampling date as the repeated random variable. The second experiment was analyzed with block, litter distribution, particle size, and sampling date as the independent fixed variable with sampling date the repeated measure. We analyzed Experiment #3 in the same way as Experiment #1. We used Akaike's information criteria (Akaike 1974) to choose a first-order heterogeneous autoregressive (ARH) covariance structure to model the repeated measure variance components using SAS mixed model procedures (Littell et al. 2005) in both experiments. Where independent variable interactions were significant ($\alpha < 0.05$), the interacting variables were analyzed separately by sampling date. Multiple comparisons within sampling dates were conducted using the Tukey-Kramer protection procedures in SAS. Differences were consider significant at the $\alpha = 0.05$ level for all ANOVAs.

RESULTS

Experiment #1: Litter Aggregation and Soil Moisture

Litter decomposition in response to aggregation was dependent on the soil WFPS (aggregation*WFPS; P<0.001, Table 3.1 and Figures 3.2 and 3.3). The more moist soil (80% WFPS) inhibited litter decomposition relative to the 50% WPPS soil at all sampling dates (P<0.0001, Table 3.2) and the magnitude of this difference decreased with incubation time (WFPS*date; P<0.0001, Table B.1).

At 50% WFPS, litter decomposition through time varied with litter aggregation (aggregation*date; P<0.0001, Table 3.3). Initially (first two sampling dates), the uniformly distributed litter decomposed at a greater rate than the

average of the aggregated litter ($P<0.05$, Table 3.4 and Figure 3.2). This was followed by an abrupt switch were the aggregated litter began decomposing more rapidly than the uniform distribution at 5 and 7 days into the incubation ($P<0.0001$, Table 3.4). On day 18, litter distributed into 9 patches was decomposing at a more rapid rate than any other distribution. Twenty-three days into the incubation, the 3 patch treatment was decomposing the fastest, 9 patches and uniform distributions were decomposing at an intermediate rate, and the single large patch treatment was decomposing at the slowest rate ($P<0.05$, Table 3.4).

In the moister soil (80% WFPS), litter decomposition was consistently higher in response to the uniform distribution than the aggregated distributions (aggregation*date; $P=1$) (uniform versus aggregated contrast; $P<0.0001$, Figure 3.3).

Nitrous oxide fluxes in response to litter aggregation was dependent on soil WFPS and varied across sampling date (Aggregation*WFPS*date; $P>0.0001$, Table 3.5 and Figures 3.2 and 3.3). The 80% WFPS soil treatment produced a wider range of N_2O fluxes (0.5 to 5000 μg N m^{-2} h^{-1}) than the 50% WFPS treatment (0.9 to 480 μg N m^{-2} h^{-1}). The highest N_2O flux rates were found between 10-15 days into the incubation for the 50% WFPS and at the first sampling date for the 80% WFPS soils. In both soil moisture treatments, N_2O flux response to litter aggregation was dependent on when the flux was measured (Table 3.6 and 3.7).

At 50% WFPS, N_2O fluxes from the uniform litter distribution were highest during the first two samplings and consistently lower than or indistinguishable from the patchy litter distribution treatments throughout the incubation (Table 3.8 and Figure 3.2). Among the patchy litter distributions at 50% WFPS there was considerable variation in the N_2O fluxes throughout the incubation (Figure 3.2 and Table B.6). Statistical differences occurred among the litter aggregation treatments at 2 days and 7 to 26 days into the incubation under 50% WFPS (Figure 3.2). The mean N_2O flux from the three patchy litter distribution was greater than the uniform distribution at days 3, 5 and 9 through 26 of the incubation in the 50% WFPS treatment.

In the moister soil, N_2O fluxes decreased with time of incubation (Figure 3.3 and Table 3.8). At this soil moisture, the uniform distribution emitted more N_2O on the last three sampling dates ($P>0.05$, Table 3.9) than the mean of the patchy litter distributions and was similar across all other sampling dates.

Experiment #2: Litter Particle Size and Aggregation

The litter decomposition rate in response to aggregation and particle size varied with sampling date (Aggregation*Date; $P<0.0001$ and Size*Date; $P<0.0001$, Table 3.10). Within 20 hours of adding water to these containers the decomposition rates of the uniformly distributed litter were higher than the patchy distributions ($P<0.01$, Figure 3.4 and Table 3.11). By the fourth day of incubation this trend was reversed with the patchy litter distribution decomposing more rapidly than the uniform litter distribution ($P<0.05$). This pattern continued through the day 14 with the exception of no differences on day 7 (Figure 3.4).

On the last two sampling dates litter distribution did not affect the decomposition rate. The litter particle size affected decomposition on four dates. On the sixth day of the incubation the finely ground litter decomposed more rapidly than the coarse litter particles ($P<0.05$). This trend was observed again during the last three sampling dates ($P<0.05$).

The patterns of nitrous oxide flux in response to litter aggregation and particle size were more complex than were CO_2 flux patterns (Aggreg.*Size*Date; $P<0.0001$, Table 3.12). For seven of the 9 sampling dates interactions between the two main factors were significant (Figure 3.3 and Table 3.13). Initially N_2O fluxes from the uniformly distributed coarse size litter were higher than from the uniformly distributed fine particles ($P>0.05$) and the N_2O fluxes from the patchy distributed litter were intermediate (Figure 3.4 and Table 3.13). By the fourth day of the incubation the patches of litter were emitting more N_2O than the uniform litter (Table 3.13). During the last four samplings N_2O fluxes were greatest from the fine sized aggregated litter, intermediate from the coarse aggregated litter and least from the uniform litter distributions regardless of particle size (Table 3.13).

Experiment #3: Litter Aggregation with Growing Plants

Nitrous oxide fluxes were highest at the beginning of the sampling period and varied in response to litter distribution (Aggregation*Date; $P<0.001$, Table 3.14). Fifteen and 20 days after litter application on DOY 160 and 165, N_2O emissions were 4.1 and 3.2 times greater, respectively, from aggregated litter

than uniformly distributed litter (Figure 3.6). In contrast on DOY 181, N_2O fluxes were 1.9 times greater from uniformly distributed litter than aggregated litter.

DISCUSSION

Experiment #1: Litter Aggregation and Soil Moisture

Role of aggregation in litter decomposition

The influence of litter aggregation on the decomposition rate of finely-ground red clover shoots was dependent on soil moisture content. Litter aggregation at optimal soil moisture for microbial activity (50%WFPS) had significant short term (0-10 days of incubation) effects on litter decomposition rate (Figure 3.2); in the longer term however, these effects were less important (Aggregation, $P<0.66$; Table 3.3). These short term effects were marked by an initial inhibition of decomposition in the aggregated litter relative to the uniform distribution. This pattern was more apparent as the aggregate size increased suggesting that extent of litter aggregation was important. By day 5 of the incubation the trend had switched such that the most highly aggregated litter was decomposing at the most rapid rate, the intermediate sized litter aggregates had intermediate decomposition rates, and the uniform litter distribution had the lowest decomposition rate. In the more moist soil (80% WFPS), the uniformly distributed litter consistently decomposed more rapidly than the aggregated litter (Figure 3.3). Thus increasing the WFPS eliminated the dynamic pattern observed across the aggregation at optimal soil moisture and caused the uniform litter distribution to decompose at the greatest rate.

The contrast between the CO_2 fluxes from the two soil moisture contents across the litter aggregation gradient is consistent with the hypothesis that O_2 diffusion initially limited microbial oxidation of the litter within the aggregates. Magid et al (2006) observed a similar decomposition pattern in response to layered maize stalks and sheep manure versus a uniform distribution at optimal soil moisture. They hypothesized that this pattern was due to N limitation in the litter that is intensified when litter is in layers as opposed to uniformly distributed. This idea that the strength or concentration of a resource sink is related to the concentration gradient of that limiting resource surrounding that sink is supported by spatial resource limitation models (Myrold and Tiedje 1985) and empirical measurements of gradients in C, N, and microbial biomass surrounding decomposition plant litter (Gaillard et al. 1999). Thus the average inorganic N concentration experienced by very small patches, i.e. particles uniformly distributed, can be greater than that of large patches per unit of metabolically available C.

The temporal differences observed in our data along the aggregation gradient at optimal soil moisture may be due to N limitation; however, the clover litter that we used has a low C:N ratio (12.9) and N limitations should diminish as soil moisture increases. Although it is possible that the aggregated litter was N limited at 50% WFPS and all of the litter distributions were O_2 limited at 80% WFPS. Overall our results suggest that aggregation plays a significant temporary yet minor role in regulating the decomposition of an N-rich labile plant litter.

Nitrous oxide response to litter aggregation

Nitrous oxide fluxes in response to litter aggregation were dependent on the sampling date and soil WFPS. At 50% WFPS, N_2O fluxes from aggregated litter differed from uniformly distributed litter on 8 of 13 sampling dates and only on the first sampling date did the uniform distribution emit more N_2O than the aggregated litter (Figure 3.3). The low levels of N_2O emitted from all of the litter treatments under 50%WFPS conditions between 5 and 7 days into the incubation are likely related to the high rates of CO_2 flux at that time. This depression in N_2O fluxes may be the result of a NO_3^- limitation and due to two possible mechanisms: microbial inorganic N immobilization or N_2O reduction to N_2. From our data it is unclear which mechanism may be more important.

Following the dip in N_2O production the aggregated litter began emitting much more N_2O than the uniform litter distribution for about 15 to 18 days. In general, the more aggregated the litter the more N_2O was emitted. Across the 39 day incubation, the average N_2O flux rates were highest from the two most aggregated litter distributions of 1 and 3 patches (154 and 146 μg N m^{-2} h^{-1}, S.E.=9, respectively), intermediate from 9 patches (98 μg N m^{-2} h^{-1}) and lowest from the uniformly distributed litter (22 μg N m^{-2} h^{-1}). From these results we conclude that aggregation of N-rich labile plant litter has a substantial influence on the N_2O emissions. Furthermore, the degree or intensity of aggregation appears to be the primary factor controlling the magnitude of N_2O emitted.

Overall the results of this experiment indicate that spatial aggregation of labile organic matter in the form of red clover litter can temporally affect

decomposition and have substantial effects on N_2O emissions than can be related to the intensity of the aggregation.

Experiment #2: Litter Particle Size and Aggregation

The interaction of particle size and aggregation on decomposition

There was general agreement in patterns of both CO_2 and N_2O fluxes between Experiments #1 and #2. The litter distribution effect dominated the early part of the decomposition pattern, whereas following about 14 days into the incubation particle size became a more significant factor with the coarsely-chopped litter decomposing at a slower rate. There are numerous studies demonstrating that finely grinding plant litter removes some of the physical protection against microbial decomposition provided by cellulose imbedded lignin. Our data suggest that during the initial phase of decomposition litter aggregation can provide a functionally similar degree of protection. Overall these results demonstrate that aggregation affects litter decomposition regardless of the litter particle size.

N_2O emissions from aggregated litter of two particle sizes

The patterns of N_2O flux were more complex than the CO_2 flux patterns. The particle size does not appear to influence N_2O fluxes from the uniformly distributed litter; however, when aggregated the same particle sizes behaved differently. Through the first 8 days of incubation the N_2O emissions from fine and coarse aggregated litter was similar and then starting about day 9 the finely-ground aggregated litter began to emit N_2O at an accelerated rate similar to the Experiment #1.

Experiment #3: Litter Aggregation with Growing Plants

The results of this field experiment concur nicely with those of the previous two incubation experiments and provide additional support that litter aggregation influences N_2O emissions for about the first 25 to 35 days following incorporation into the soil. The maize plants in this study were planted from seed 5 days following litter application (DOY 150) and thus were likely too small to have a substantial effect on the microbial processes that generate N_2O until after the aggregation effect discontinued. This conclusion is also supported by the observation that N_2O fluxes from unplanted and planted containers were similar in this experiment (data not shown).

CONCLUSIONS

The aggregation of red clover litter in soil had a transient effect on decomposition patterns under 50% WFPS. In wetter soils (80 %WFPS) this short-term effect was eliminated, potentially indicating that O_2 diffusion into the litter aggregates was regulating the decomposition of the aggregated litter. Litter aggregation had substantial effects on N_2O emissions regardless of the litter particle size or if plants were growing in the soil. In fact, the more intensely the litter was aggregated the greater were the N_2O fluxes. This resulted in the most aggregated litter treatment of our experiments emitting 7 times more N_2O than uniformly distributed litter under 50% WFPS.

This observation that litter distribution alters N_2O fluxes has important implications for how we estimate N_2O emissions for greenhouse gas inventories and mitigation strategies. For example, the IPCC inventory standards estimate

that 1.25% of N applied to agricultural soils is lost as N_2O. Here we show that the same quantity of litter-N can emit a 7-fold difference in N_2O simply depending on its horizontal distribution in soil. Managing agricultural plant litter in a spatially explicit manner may offer an effective strategy for migrating N_2O emissions.

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Table 3.1 Analysis of variance of CO_2 flux in response to litter aggregation and soil moisture as water-filled pore space (WFPS) across a 39 d incubation at 22°C.

Source of variation	Num DF†	Den DF	F Value	Pr > F
Block	4	338	12.17	<.0001
Aggregation	3	338	21.38	<.0001
WFPS	1	338	553.48	<.0001
Aggreg. x WFPS	3	338	18.89	<.0001
Sampling date	12	338	81.85	<.0001
Aggreg. x date	36	338	0.34	0.9999
WFPS x date	9	338	7.82	<.0001
Aggreg. x WFPS*date	27	338	0.29	0.9999

† indicates the numerator (Num) and denominator (Den) degrees of freedom.

Table 3.2 Interaction slice of CO_2 flux for sampling date by soil moisture treatment.

Main effect slice	Date	Num DF†	Den DF	F Value	Pr > F
WFPS x Sampling date					
	2.0	1	338	42.10	<.0001
	3.0	1	338	42.57	<.0001
	5.0	1	338	116.2	<.0001
	7.2	1	338	78.65	<.0001
	9.0	1	338	80.95	<.0001
	11.0	1	338	77.98	<.0001
	15.0	1	338	64.89	<.0001
	17.8	1	338	66.92	<.0001
	22.9	1	338	18.81	<.0001
	36.0	1	338	26.30	<.0001

† indicates the numerator (Num) and denominator (Den) degrees of freedom.

Table 3.3 Analysis of variance of CO_2 flux response to litter aggregation at 50%WFPS across a 39 d incubation at 22°C.

Source of variance	Num DF†	Den DF	F Value	Pr > F
Block	4	191	4.16	0.0030
Aggregation	3	191	0.53	0.6636
Sampling date	12	191	767.35	<.0001
Aggreg. x date	36	191	3.90	<.0001

† indicates the numerator (Num) and denominator (Den) degrees of freedom.

Table 3.4 Analysis of variance of CO_2 flux rate from 50% WFPS treatment by sampling date and aggregation treatment means with multiple comparisons adjusted by Tukey-Kramer procedure.

days of incubation	Aggregation Effect	Uniform vs. Aggregation	1 - patch		3 - patches		9 - patches		Uniform	
	P>F		Treatment Means							
	3,12†	1,12								
2.0	0.0657	0.0397	165.0	ns	246.8	ns	289.1	ns	388.5	ns
3.0	0.0212	0.0199	222.8	b	292.3	ab	263.4	ab	322.2	a
5.0	0.0001	0.0001	533.9	a	475.4	a	475.6	a	317.3	b
7.2	0.0001	0.0001	352.2	a	334.7	ab	273.8	b	220.9	c
9.0	0.0095	0.6073	251.4	a	198.8	b	197.8	b	213.8	ab
11.0	0.0585	0.2296	183.8	ns	161.8	ns	160.6	ns	178.9	ns
15.0	0.3053	0.3923	127.6	ns	113.7	ns	138.7	ns	118.7	ns
17.8	0.0001	0.1513	78.5	b	70.7	b	105.3	a	77.5	b
22.9	0.0159	0.3794	46.0	b	62.6	a	59.1	ab	54.2	ab
26.0	0.4054	0.8633	67.9	ns	78.6	ns	67.0	ns	69.7	ns
29.9	0.8751	0.7791	50.1	ns	51.1	ns	47.3	ns	48.2	ns
36.8	0.7851	0.5748	34.2	ns	36.2	ns	35.3	ns	33.9	ns
38.8	0.2745	0.5482	25.8	ns	29.1	ns	27.8	ns	28.6	ns

† All sampling dates had 5 replicates except the first sampling date which had two replicates.
Different letters after each treatment mean flux within sampling date indicates statistical significance ($\alpha = 0.05$) by the Tukey-Kramer multiple comparison procedure.

Table 3.5 Analysis of variance of N_2O flux in response to litter aggregation and soil moisture across a 39 d incubation at 22°C.

Source of variance	Num DF†	Den DF	F Value	Pr > F
Block	4	340	5.29	0.0004
Aggregation	3	340	24.42	<.0001
WFPS	1	340	128.52	<.0001
Aggreg. x WFPS	3	340	57.05	<.0001
Sampling date	12	340	62.09	<.0001
Aggreg. x date	36	340	4.05	<.0001
WFPS x date	9	340	45.31	<.0001
Aggreg. x WFPS x date	27	340	5.70	<.0001

† indicates the numerator and denominator degrees of freedom.

Table 3.6 Analysis of variance of N_2O flux in response to litter aggregation with 50% WFPS soil across repeated sampling of a 39 d incubation at 22°C.

Source of variance	Num DF†	Den DF	F Value	Pr > F
Block	4	192	3.65	0.0069
Aggregation	3	192	97.06	<.0001
Sampling date	12	192	74.31	<.0001
Aggreg. x date	36	192	14.79	<.0001

† indicates the numerator (Num) and denominator (Den) degrees of freedom.

Table. 3.7 Analysis of variance of N_2O flux in response to litter aggregation with 80% WFPS soil across repeated sampling of a 39 d incubation at 22°C.

Source of variance	Num DF†	Den DF	F Value	Pr > F
Block	4	144	27.23	<.0001
Aggregation	3	144	22.45	<.0001
Sampling date	9	144	84.95	<.0001
Aggreg. x date	27	144	2.03	0.0043

† indicates the numerator (Num) and denominator (Den) degrees of freedom.

Table 3.8. Analysis of variance of N_2O flux rate from the 55% WFPS treatment by sampling date and aggregation treatment means with multiple comparisons adjusted by Tukey-Kramer procedure.

days of incubation	Aggregation Effect	Uniform vs. Aggregation	1 - patch		3 - patches		9 - patches		Uniform	
	P>F		Treatment Means							
	3,12†	1,12								
2.0	0.1255	0.0794	89.0	ns	193.8	ns	176.5	ns	74.2	ns
3.0	0.0002	0.0016	340.6	a	306.6	a	95.2	b	77.5	b
5.0	0.0721	0.0395	23.2	ns	16.6	ns	14.7	ns	29.1	ns
7.2	0.0255	0.8488	8.6	b	73.5	a	48.9	a	27.0	ab
9.0	0.0001	0.0001	139.4	b	317.9	a	297.2	ab	30.6	c
11.0	0.0001	0.0001	480.1	a	423.7	a	299.3	a	17.8	b
15.0	0.0001	0.0001	449.7	a	293.7	ab	188.2	b	3.7	c
17.8	0.0001	0.0001	323.2	a	70.5	b	110.5	b	3.5	c
22.9	0.0001	0.0001	52.1	a	89.8	a	13.6	b	0.9	c
26.0	0.0001	0.0001	51.2	a	47.6	a	7.4	b	2.0	b
29.9	0.1975	0.1875	29.0	ns	30.3	ns	8.1	ns	8.4	ns
36.8	0.2224	0.1725	13.4	ns	16.2	ns	5.3	ns	5.7	ns
38.8	0.4719	0.9641	6.2	ns	14.5	ns	5.3	ns	7.8	ns

† indicates the numerator (Num) and denominator (Den) degrees of freedom, respectively.

Table 3.9 Analysis of variance of N_2O flux rate from the 80% WFPS treatment by sampling date and aggregation treatment means with multiple comparisons adjusted by Tukey-Kramer procedure.

days of incubation	Aggregation Effect	Uniform vs. Aggregation	1 - patch		3 - patches		9 - patches		Uniform	
	P>F		Treatment Means							
	3,12†	1,12								
2.0	0.0815	0.0612	2596	ns	2408	ns	537.6	ns	4988	ns
3.0	0.0777	0.4490	1057	ns	690	ns	271.9	ns	1622	ns
5.0	0.0442	0.4558	64.8	ns	37.3	ns	7.1	ns	129.6	ns
7.2	0.0099	0.7731	28.6	a	23.1	ab	5.6	b	28.7	a
9.0	0.0510	0.5646	22.8	ns	12.8	ns	3.1	ns	26.3	ns
11.0	0.4094	0.3480	10.0	ns	3.8	ns	1.3	ns	27.7	ns
15.0	0.2032	0.0785	3.6	ns	1.6	ns	0.8	ns	10.4	ns
17.8	0.0449	0.0141	3.2	ns	0.7	ns	0.5	ns	9.4	ns
22.9	0.0047	0.0005	0.9	b	1.2	b	1.9	b	14.1	a
26.0	0.0005	0.0001	2.0	b	1.2	b	2.0	b	14.1	a

† indicates the numerator and denominator degrees of freedom, respectively.

Table 3.10 Analysis of variance of CO_2 flux in response to litter aggregation and litter particle size across a 18 d incubation at 23°C.

Source of variation	Num DF†	Den DF	F Value	Pr > F
Block	3	105	3.17	0.0275
Particle size	1	105	0.03	0.8592
Aggregation	1	105	0.69	0.4067
Aggreg. x Size	1	105	1.69	0.1966
Sampling date	8	105	421.92	<.0001
Aggreg. x Date	8	105	5.79	<.0001
Size*Date	8	105	6.63	<.0001
Aggreg. x Size x Date	8	105	1.97	0.0579

† indicates the numerator (Num) and denominator (Den) degrees of freedom, respectively

Table 3.11 Analysis of variance of CO_2 flux rate in response to litter particle size and aggregation treatment by sampling date and means with multiple comparisons adjusted by Tukey-Kramer procedure.

Source of variance	Particle Size	Aggregation	Aggreg. x Size	Fine				Coarse				
	P>F			Uniform		3 - patches		Uniform		3 - patches		Control§
days of incubation	1,9†	1,9	1,9	Treatment Means‡								
0.9	0.6579	0.0053	0.2767	479	a	199	b	446	ab	292	ab	112
2.2	0.3027	0.1535	0.6260	869	ns	649	ns	718	ns	602	ns	109
4.0	0.4084	0.0488	0.9750	463	ns	569	ns	491	ns	634	ns	67
6.2	0.0162	0.0001	0.0504	252	c	377	ab	314	b	390	a	57
6.9	0.1762	0.6727	0.5549	145	ns	165	ns	173	ns	220	ns	85
8.7	0.1688	0.0006	0.0105	138	b	152	b	128	b	182	a	30
13.9	0.0082	0.0136	0.1094	113	a	120	a	88	b	111	ab	28
15.9	0.0049	0.2288	0.6344	90	ab	100	a	75	b	79	ab	24
17.9	0.0058	0.4598	0.2786	91	ab	103	a	77	ab	76	b	28

† indicates the numerator and denominator degrees of freedom.
‡ different letters following means indicate significant differences within sampling dates.
§ the no-litter control treatment was not included in the ANOVA

Table 3.12 Analysis of variance of N_2O flux in response to litter aggregation and litter particle size across a 18 d incubation at 23°C.

Source of variation	Num DF†	Den DF	F Value	Pr > F
Block	3	105	5.51	0.0015
Particle size	1	105	16.29	0.0001
Aggregation	1	105	409.49	<.0001
Aggreg. x Size	1	105	184.62	<.0001
Sampling date	8	105	61.82	<.0001
Aggreg. x Date	8	105	32.69	<.0001
Size x Date	8	105	31.63	<.0001
Aggreg. x Size x Date	8	105	14.20	<.0001

† indicates the numerator (Num) and denominator (Den) degrees of freedom, respectively.

Table 3.13 Analysis of variance of N_2O flux rate in response to litter particle size and aggregation treatment by sampling date and means with multiple comparisons adjusted by Tukey-Kramer procedure.

Source of variance	Particle Size	Aggreg.	Aggreg. x Size								
				Fine				Coarse			
	P>F			Uniform		3 - patches		Uniform		3 - patches		Control
Degrees of freedom days of incubation	1,9†	1,9	1,9								
				Treatment Means							
0.9	0.0348	0.7589	0.0404	41.8	b	67.4	ab	135.3	a	69.9	ab	4.2
2.2	0.3355	0.1734	0.6971	124.5	ns	168.9	ns	90.3	ns	137.6	ns	11.6
4.0	0.0008	0.0001	0.0090	12.2	c	170.0	a	58.9	b	219.7	a	4.8
6.2	0.3546	0.0001	0.0001	7.2	c	77.0	a	30.0	b	29.5	b	0.4
6.9	0.3953	0.3515	0.0586	6.3	ns	32.6	ns	22.7	ns	14.9	ns	10.0
8.7	0.9208	0.0001	0.0001	5.9	c	48.8	a	15.6	b	23.9	b	0.1
13.9	0.0004	0.0001	0.0012	1.9	c	373.0	a	1.2	c	28.9	b	0.1
15.9	0.0001	0.0001	0.0001	2.6	c	232.1	a	4.9	c	12.0	b	0.8
17.9	0.0001	0.0001	0.0001	0.3	c	153.1	a	5.5	b	7.6	b	2.1

† indicates the numerator and denominator degrees of freedom, respectively.

Table 3.14 Analysis of variance of N_2O flux in response to litter aggregation with a growing maize plant across repeated sampling.

Source of variance	Num DF†	Den DF	F Value	Pr > F
Block	3	110	2.21	0.092
Aggregation	4	110	5.64	0.0004
Sampling date	7	110	28.2	<.0001
Aggreg.*date	28	110	2.88	<.0001

† indicates the numerator (Num) and denominator (Den) degrees of freedom, respectively.

Table 3.15 Analysis of variance of N_2O flux in response to litter aggregation and growing plants by sampling date and means with multiple comparisons adjusted by Tukey-Kramer procedure.

Source of variance	Aggregation										
	4,12†	8 - patches		24 - patches		72 - patches		Uniform		Control	
Day of Year	P>F	Treatment Means‡									
160	0.0064	47.6	abc	164.2	ab	174.3	a	31.2	bc	11.8	c
165	0.012	65.5	ns	87.5	ns	48.1	ns	21.2	ns	13.3	ns
170	0.021	70.6	a	19.7	ab	19.1	ab	17.3	ab	14.3	b
181	0.037	12.4	ns	5.3	ns	12.4	ns	19.5	ns	5.5	ns
200	0.495	5.6	ns	5.8	ns	16.6	ns	11.8	ns	14.5	ns
208	0.509	5.9	ns	9.3	ns	3.6	ns	8.9	ns	7.5	ns
217	0.678	0.6	ns	0.2	ns	1.4	ns	1.6	ns	2.1	ns
233	0.469	1.2	ns	11.9	ns	10.5	ns	18.5	ns	6.9	ns

† indicates the numerator and denominator degrees of freedom.
‡ different letters following means indicate significant differences within sampling dates.

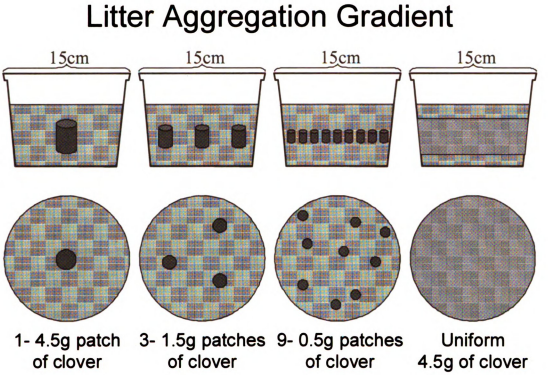

Figure 3.1 Spatial treatment layout of the litter aggregation gradient. The top row shows a vertical profile of the litter distribution placement in the 2.6-L containers. The bottom row of depicts a top-down view of the horizontal distribution of the litter.

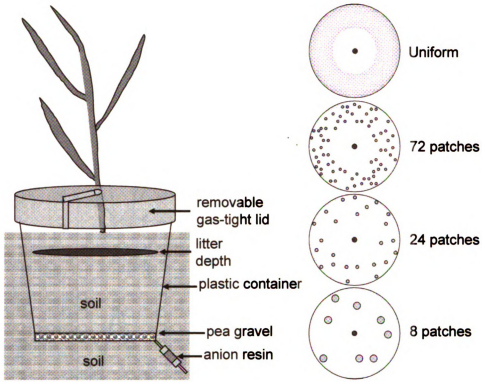

Figure 3.2 Schematic depiction of the spatial layout of litter distribution treatments conducted in 50-L plastic containers used in the field experiment. Containers were set into holes in a field and surrounded by maize plants. The left panel contains a side-cut view of the container components and vertical layout. The right panel is a top-down view illustrating the spatial distribution of the litter treatments.

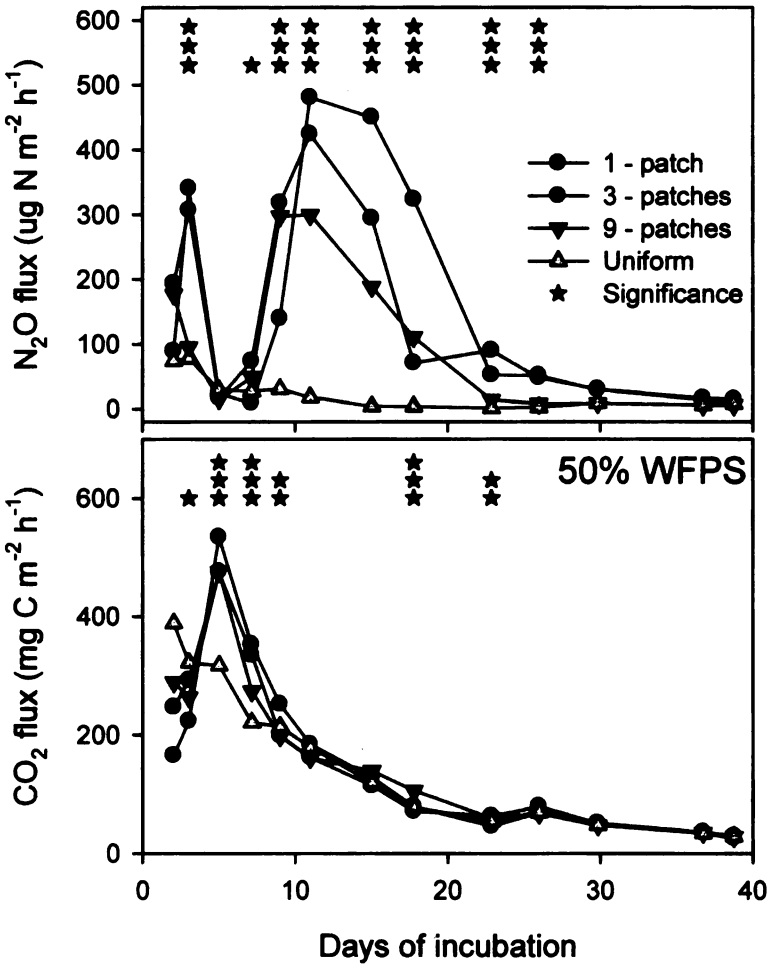

Figure 3.3 CO_2 and N_2O flux response to litter aggregation at 50% WFPS during a 39d incubation. The results of the ANOVA contrast comparing the treatment means of all the aggregated litter to the uniformly distributed litter are presented across the top of each graph. *, **, and *** indicate statistical significance at P= 0.05, 0.01, and 0.001 levels, respectively for the contrast.

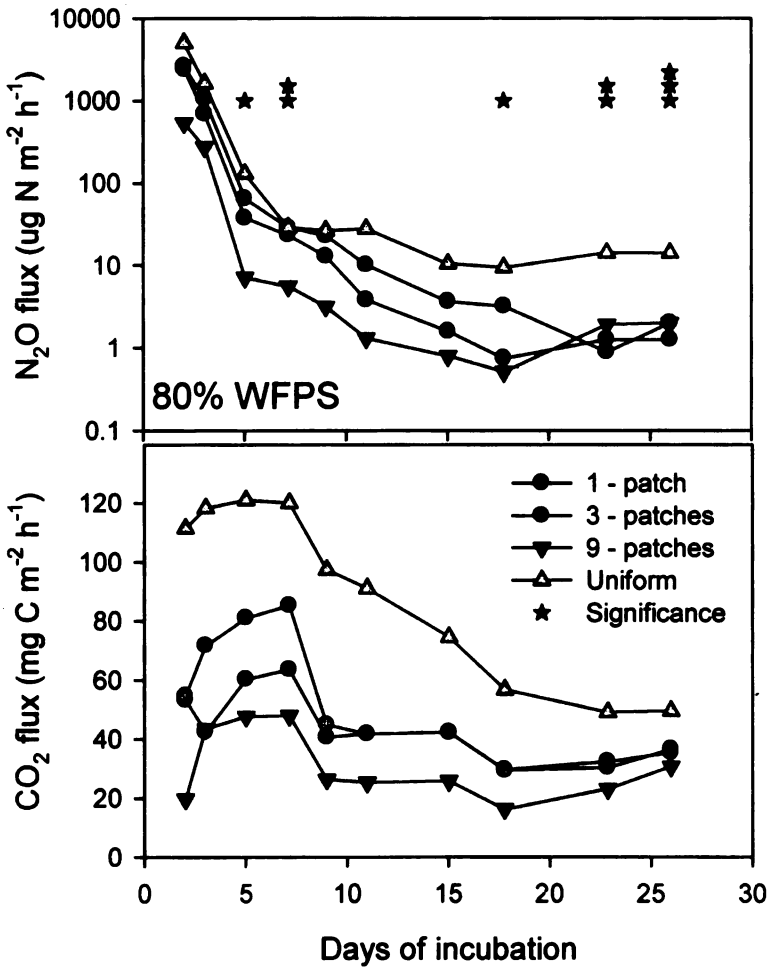

Figure 3.4 CO_2 and N_2O flux response to litter aggregation and at 80% WFPS during a 38d incubation. The results of the ANOVA contrast comparing the treatment means of all of the aggregated litters to the uniformly distributed litter are presented across the top of the N_2O flux graph. ⋆, ⋆⋆, and ⋆⋆⋆ indicate statistical significance at the 0.05, 0.01, and 0.001 levels, respectively for the contrast. The repeated measures ANOVA indicated that the CO_2 flux was greater ($P<0.05$) in the uniform distribution than the mean of the aggregated litter across the entire incubation.

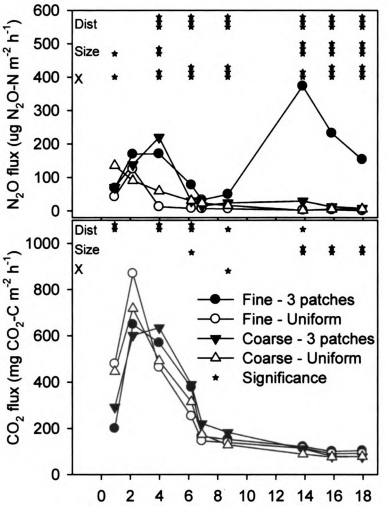

Figure 3.5 CO_2 and N_2O flux response to litter aggregation and litter particle size during an 18d incubation. The results of the ANOVA are presented across the top of each graph. *, **, and *** indicate statistical significance at the 0.05, 0.01, and 0.001 levels, respectively for the two main effects, Distribution (Dist) and Particle Size (Size) and their interaction term (X).

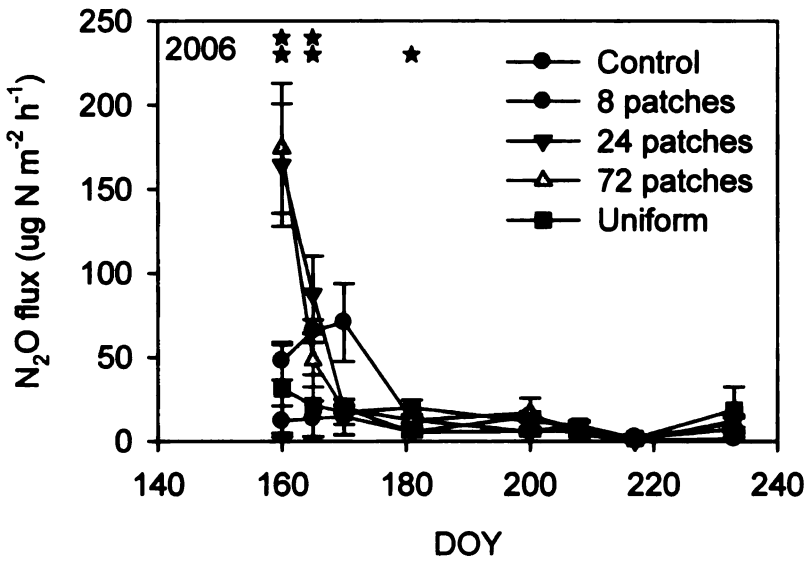

Figure 3.6 Nitrous oxide flux response to litter aggregation with growing maize plants in 50-L containers. The results of the ANOVA are presented across the top of the graph. * and ** indicate statistical significance at the 0.05 and 0.01 levels, respectively, for the contrast between aggregated and uniformly distributed litter.

Chapter 4. Aggregated soil resources enhance primary productivity

INTRODUCTION

Variation in plant growth responses to soil resource heterogeneity is likely attributable to soil resource characteristics, experimental conditions, and plant root foraging traits (Fitter et al. 2000, Hodge 2006). For example, the spatial aggregation of phosphate fertilizers into patches or strips almost always improves P use efficiency and plant productivity relative to the same quantity of P distributed uniformly in soil (Kume et al. 2006). Root proliferation into patches of phosphates increases uptake efficiencies because a relatively small mass of roots can satisfy plant P uptake demands instead of the entire root system foraging for P (Zhu et al. 2005, Kume et al. 2006). In contrast, the spatial aggregation of nitrate (NO_3^-), a more mobile plant nutrient, also often stimulates root proliferation (Drew 1975) but with inconsistent effects on productivity (Robinson 1994). One reason for this may be that NO_3^- may act as a signal to initiate root proliferation (Zhang et al. 2007) because of its association in unfertilized systems with decomposing organic matter; however, if the NO_3^- is not associated with a source of more NO_3^- (i.e., organic matter) then the proliferation response may require more plant resources than are obtained from the NO_3^- patch. Hodge et al. (1999) found support for this idea when root proliferation of two competing plants was only related to plant N capture from patches of complex organic substrate (plant litter), not patches of inorganic N.

Many plant species exhibit compensatory root growth in response to soil resource heterogeneity (Robinson 1994) – root biomass increases in areas of enriched soil nutrients and decreases in areas of scarce resources. This can result in higher nutrient uptake efficiency if resources are acquired more efficiently from patches of high resource concentration because more nutrients are captured with the same quantity of root biomass or less root biomass is required to meet the nutritional demands of the plants. Thus optimization principles suggest that the root to shoot biomass ratio (R:S) should decrease in plant-soil systems that are acquiring soil nutrients more efficiently (Hutchings and John 2004). Because of its easily confirmed plastic R:S, the clonal plant *Glechoma hederacea* (Birch and Hutchings 1994) has been used to demonstrated the predictions of this hypothesis most clearly; however, tests of this hypothesis with non-clonal plants have yielded mixed results (Robinson 1994).

In agricultural ecosystems, a shift in the R:S due to changes in nutrient uptake efficiency will be manifested in altered grain yield. We undertook this study to address the following questions: 1) does the aggregation of labile organic N influence the growth of individual maize plants in a system limited by N availability, 2) is R:S and belowground C allocation altered by resource aggregation, and 3) does the distribution of aggregated resources influence maize productivity?

MATERIALS AND METHODS

We conducted two studies at the W.K. Kellogg Biological Station (KBS) in Southwest Michigan (42° 24'N, 85°24'W, elevation 288 m). In the first study, we examined the effect of clustered plant litter aggregation on maize productivity. In contrast, the second experiment studied the influence of randomly distributed litter aggregates on aboveground and belowground plant responses. A 1:1 mix of coarse sand and a composite of soil taken from the surface 0.4 m soil of a field on the KBS Long-Term Ecological Research site was used in each experiment. Soils of the Kalamazoo (fine-loamy, mixed, mesic Typic Hapludalfs) and Oshtemo (coarse-loamy, mixed mesic Typic Hapluadalfs) series co-occur on the site and were both present in the soil used in this experiment. The soil was excavated from the field on May 19, 2005, mixed with an end-loader, placed in a tarp-covered pile to air dry, power sieved through a 12 mm screen, combined with coarse sand in a 250 L mixer, and stored in a common covered pile until use.

Clustered Litter Distribution – Experiment #1

The clustered litter distribution experiment was laid out in a Randomized Complete Block Design (RCBD) with 6 soil amendment treatments, 5 sampling dates, and 4 replicates per block. The final sampling date included an additional no N added control (–N) treatment for a total of 124 experimental units. Each experimental unit was a 50-L black plastic container filled with the soil mix, amendment, and a single maize plant (Pioneer ® 35Y54) (Figure 4.1). Litter species (*Trifolium pratense* and *Secale cereale*) and plant litter spatial

distribution in the soil were the manipulated factors plus $NaNO_3$ fertilizer control (control +N) and no N added control (control -N) treatments.

Litter consisted of *Trifolium pratense* (red clover) and *Secale cereale* (rye) (Michigan State Seed Solutions, Grand Ledge, MI, USA) produced in sand in a greenhouse, fertilized with a modified complete Hoagland's nutrient solution (Hewitt 1966), and harvested before initiation of the reproductive phase. The shoots were cut at the sand surface, dried for 4-5 days at 55°C, coarsely-chopped to pass a 10 mm screen with a Wiley® mill, and then finely-ground to pass a 1 mm screen in a Cyclotec® 1093 sample mill. Eight subsamples of each finely-ground litter were analyzed for C and N content with a Costech ECS 4010 CHN elemental analyzer. The red clover litter contained 410 g C kg litter^{-1} and 29.8 g N kg litter^{-1}, for a C:N ratio of 13.7, and the rye contained 392 g C kg litter^{-1} and 16.1 g N kg litter^{-1}, for a C:N ratio of 24.4. The red clover litter was aggregated into 8 or 32 patches or distributed uniformly into the soil, whereas the rye was aggregated into 8 patches or distributed uniformly (Figure 4.1).

Litter application

Litter distribution treatments were constructed by placing a 150 mm diameter x 150 mm length polyvinylchloride (PVC) cylinder flat onto a 50 x 185 x 185 mm square Teflon® coated baking dish that had one side removed. Into the PVC cylinder we added 600 mL of soil mix, then a template for the aggregated litter treatments. The template was a 145 mm diameter circular sheet of acrylic with four 21.3 mm or sixteen 8.3 mm diameter plastic syringe cylinders (without the needle hub end) adhered perpendicular to the sheet. We used the syringe

plungers to displace a consistent volume of soil within the syringe cylinders before adding the litter. Around the template we added 300 mL of soil. We pushed the plungers down into the cylinders as the template was raised such that the litter remained in the soil as individual column shaped patches just below the surface of the soil. After the template was removed we added 400 mL of soil on top of the litter. Each patch of litter had the same mean depth in the soil (Figure 4.1). The uniform treatment was constructed by first adding 300 mL of soil to the containers, then 600 mL of a litter and soil mixture that was then topped off with 400 mL of additional soil. The red clover and rye litter added to each PVC cylinder was 28.87 g (sixteen 1.80 g or four 7.22 g patches, or uniformly 28.87g) and 36.02 g (4-9.00 g or uniformly 36.02 g), respectively.

Field containers

We prepared 50-L (370 mm in depth, 390 mm bottom diameter and 440 mm top diameter) containers for this experiment by directing all of the drainage through a single outlet and adding 3 L of 8 to 10 mm diameter gravel to the bottom of the container to aid drainage. We lined the container above the gravel with a medium weight landscaping fabric to keep soil in the containers. Into each lined-container we added 40 L of soil mix and placed each container in the holes in the field that were created when the soil was originally excavated. The containers were placed in the field such that each was at least 2.5 m from others and into the soil such that the soil inside and outside the containers were approximately at the same depth. We chose this container size because it has a surface area of about 0.15 m^2 which is similar to the standard production

practices in this region of 0.14 m^2 $plant^{-1}$. The spaces between the containers were planted to the same maize hybrid; plants were thinned so as to not shade out the seedlings in the containers. We covered the drainage holes in the bottom of the containers with duct tape from the inside and outside, which proved to be an effective moisture barrier in preliminary tests. A 12 mm circular hole was cut out of the container at the bottom to allow a standard drainage. For the containers to be harvested on the second and final sampling dates were outfitted with anion exchange resin columns to capture the anions draining out of the containers (see Chapter 5).

The anion exchange columns consisted of 30 mL of Type II 16-50 mesh anion exchange resin (IONAC® A-554 Cl-form) held in a 250 mm length of 32 mm diameter clear vinyl tubing with 8 µm fiberglass (Corning Inc., Corning, NY). The columns were fitted to the drain hole on the outside of each container with vinyl fittings. We covered the opening of the drainage hole in the container with a garden hole screen (~1 mm) to prevent sediment from flowing into the column. To ensure that all of the drainage water flowed through the column, the column was secured to the container with rubber gaskets and 100% silicon rubber sealant (DAP Inc., Baltimore, MD). These containers were tilted about 1% towards the drain holes to aid drainage.

Fertilization and planting

On June 23-24, 2005, two litter-amended PVC soil cylinders were placed on the soil surface in each container; each cylinder was placed into about a third of the surface area of the container (Figure 4.1). We added an additional 4 L of

soil around each of the PVC cylinders such that the soil depth in the entire container was about 20 mm from the top of the container. The PVC cylinders were raised such that 120 mm of the cylinders were above the soil, thus leaving the amended soil in contact with the rest of the soil in the container (Figure 4.1). The PVC cylinders were left in place throughout the season to indicate the placement of the amended soil and to serve as gas flux chamber bases.

We designed this experiment to test maize N response to litter distribution and litter species in a system where N was the only soil nutrient limiting plant growth. On June 28, 2005, we added 250 mL of nutrient solution to the surface of each container. Each 250 mL dose of solution contained 0.5 P g, 1.25 K g, 0.25 S-SO4 g, 0.27 Ca g, and 0.07 Mg g. The control +N treatment was implemented on July 8, 2005 by adding 1 L of solution containing 1.6 g N as $NaNO_3$ to each container, whereas the -N and litter treatments all received 1 L of RO (reverse osmosis) water. Supplemental watering (2 L container^{-1}) occurred on August 1, 20, and 28.

On June 29, 2005, we transplanted two d old maize seedlings into the center of each container and placed 6 mm screen hardware cloth exclosures over the center of the containers until the seedlings were at the V2 growth stage (Hanway 1963) to protect the seedlings from rodent damage.

Repeated harvest

On July 6 and 20, August 3 and 23, and October 6, 2005, we harvested four replicates of each treatment. The aboveground shoots were cut at the soil surface, dried (4 days at 60°C), and weighed. We hauled the containers to a

laboratory to determine soil moisture contents in the surface 190 mm and the bottom 180 mm of each container. We sieved soil and roots were sieved to pass a 6 mm screen, weighed wet, and sub-sampled for soil moisture. We dried (4 d at 65°C) and weighed the sub-sample. On the final harvest date we harvested two containers for aboveground biomass for each experimental unit and the control -N treatment. For the control +N treatment, we separated the roots from the soil during the dry sieving process. We stored the roots in a 10% v.v. ethanol solution at 4°C until processing to remove mineral particles and then we dried (60°C for 4 days) and weighed the roots.

Random Litter Distribution – Experiment #2

This experiment utilizes the same basic litter patch size distribution as Experiment #1, but here the patches are randomly distributed throughout a similar depth of soil. Additionally, we quantified belowground biomass and root respiration as well as the aboveground response. This experiment was conducted in the 50-L containers during the 2006 growing season in the same field as above and is laid out in a RCBD with 4 replicates, 5 soil amendment treatments, and 7 sampling dates. The same soil preparation procedure and container placement in the field was used as earlier except it was not necessary to use landscape fabric. Red clover was produced in the greenhouse, as described earlier. We distributed the litter (shoots) into 8, 24, or 72 patches or uniformly in the soil except no patches were placed within the center 150 mm of the container (Figure 4.1) to avoid disturbing the litter patch when planting the

maize. The red clover litter contained 413 g C kg litter^{-1} and 31.0 g N kg litter^{-1} giving it a C:N ratio of 13.3.

Litter application

The litter application method was modified from earlier. We applied the litter directly to soil in the containers with a template that was the same diameter as the inside of the container (Figure 4.1). The number and sizes of the litter patches were eight 4.69 g patches, twenty-four 1.56 g patches, seventy-two 0.52 g patches, and the uniform distribution of 37.5 g of litter. The patches were distributed at a mean depth of 100 mm below the soil surface. The litter application process lasted three days from May 22-24, 2006.

Planting and fertilizing

On May 25, 2006, we transferred the containers to the field and on May 26 we placed the anion exchange columns on the containers to be harvested on the third and last harvest dates. We planted two maize seeds (Pioneer 35Y54) to a depth of ~50 mm into the center of the each container on May 31 and covered with hardware cloth exclosures. In between the containers we planted maize seeds at standard production densities (70,000 plants ha^{-1}). After 14 days, we thinned the plants to one plant container^{-1}. We fertilized as earlier on July 6, 2006. Supplemental watering (2 L container^{-1}) occurred on August 13 and 22.

Repeated harvests

We conducted seven repeated harvests for this experiment. Four of the harvests were used to quantify aboveground plant growth and the other three used to quantify belowground plant growth in response to the litter distribution

treatments. On June 22, July 20, August 22, and October 12, 2006, we harvested four replicate containers from each treatment and processed the shoots and the soil as in Experiment #1. To quantify belowground biomass we harvested containers on July 7, August 14, and September 26, 2006. The aboveground biomass was processed in the same manner as above, but the roots were separated from the soil through a combination of flotation and wet sieving over a 1 mm screen. The entire root system was collected into 4-L sealable plastic bags, covered with a 10% v.v. ethanol solution, and stored at 4°C. We used a secondary processing to separate the maize roots from other detritus, soil particles, and any remaining clover litter then we dried (60°C for 4 d) and weighed the roots.

Soil surface CO_2 flux

Plant C allocated belowground can have several fates including root biomass, root metabolic respiration, soil microbial biomass, soil microbial respiration, and deposition into the soil matrix. Carbon respired by plant roots and soil heterotrophs makes up most of the CO_2 that is emitted from the soil surface. To distinguish the CO_2 derived from roots versus soil organic C (SOC) oxidation we measured the CO_2 flux from containers and with and without growing maize plants (Hanson et al. 2000). To accomplish this an additional set of non-planted containers with and without uniformly distributed litter were added to each replicate. The difference between CO_2 flux from planted and non-planted containers within each replicate is our proxy for total root derived respiration.

We used removable static chambers to measure soil surface CO_2 flux (Holland et al. 1999). Each chamber lid sealed around the outside of the container and the plant stem (Figure 4.2). The chamber lids were modified 120-L refuse container and lid with a 60 mm wide slit removed from on edge to the center of the lid to accommodate the plant stem. Latex sheeting was secured to the plant stem and chamber lid to complete an air tight seal. Deployment of the chamber lid required about 3 minutes and remained on the container for a maximum of 70 minutes during the sampling. We sampled the chamber headspace by using a 10 mL plastic syringe to transfer 20 mL of gas from the chamber to 5.9 mL glass vials outfitted with rubber septa. We used an infrared gas absorption analyzer (LI-Cor 820) to determine the CO_2 gas concentration. The sample CO_2 concentrations were regressed with a linear model against sampling time to determine the gas flux rate (Holland et al. 1999).

Statistical Analysis

For Experiment #1, we used a repeated measure analysis of variance (ANOVA) for each response variable with block and soil amendment treatment (litter species, litter distribution, and controls) as independent fixed variables with sampling date as the repeated random variable. We analyzed Experiment #2 similarly with block and litter distribution as the independent fixed variables and sampling date being the repeated random variable. We used Akaike's information criteria (Akaike 1974) to choose a first-order heterogeneous autoregressive (ARH) covariance structure to model the repeated measure variance components using SAS mixed model procedures (Littell et al. 2005) in

both experiments. Data from the first sampling date of Experiment #2 was omitted from the biomass response analyses because the ARH model would not converge due to the lack of variance in biomass at this early sampling date. Soil surface CO_2 flux data were natural log transformed to meet the homogeneity of the variance assumption of ANOVA. When the interaction of main effects and sampling date were significant ($\alpha < 0.05$) the interacting variables were analyzed separately by sampling date. Single degree of freedom contrasts were used to determine effects of species identity and litter distribution for each date for Experiment #1 and to determine the effect of litter distribution for each date in Experiment #2. Differences were consider significant at the $\alpha < 0.05$ level for all ANOVAs.

RESULTS

Experiment #1: Plant Growth Response to Clustered Litter Distribution

In Experiment #1, when the amended litter was clustered within 23% of the soil surface area (Figure 4.1), the aboveground maize response to soil amendments varied with sampling date (soil amend. x date; $P<0.0001$, Table 4.1 and Figure 4.2). There were no differences in aboveground biomass until the final harvest (DOY 279) of 2005 (Table 4.2). On this date, aboveground biomass response to litter distribution was dependent on the litter species used as the soil amendment (litter species*distribution; $P<0.027$, Figure 4.3). All of the litter amendment treatments enhanced maize productivity relative to the control -N treatment and appeared to be N limited relative to the control -N treatment. The aggregation of clover litter into 8 and 32 patches enhanced aboveground

biomass by 13% (119 ± 4.9 g plant^{-1}) relative to the uniform distribution (105 ± 4.9 g plant^{-1})(Figure 4.3). The distribution of the rye litter did not affect aboveground biomass at the final sampling (P>0.49).

The root biomass in Experiment #1 varied throughout the season (Figure4.6); R:S ranged from 0.48 at the first sampling to 0.17 at the last sampling date. Soil moisture content in the surface 190 mm and in the bottom 180 mm varied from 0.13 to .07 g H_2O g soil^{-1} and 0.18 to 0.08, respectively, throughout the season, but was unaffected by soil amendment treatment on any sampling date (P>0.05) (data not shown).

Experiment #2: Plant Response to Random Litter Distribution

Growing season average aboveground biomass was greater in treatments with aggregated litter (8, 24, and 72 patches) than to uniformly distributed litter (P<0.025; Table 4.3). Most of the treatment occur during the grain filling growth stage (Figure 4.4 and Table 4.4). At physiological maturity, aboveground maize biomass response to aggregated litter (315 g container^{-1}) was similar to the control +N (P<0.061), whereas the plants growing in the containers treated with a uniform distribution were 25% smaller than the control +N (P<0.001) and 16% smaller than those in the aggregated litter treatments (P=0.025) (Figure 4.5 and Table 4.3).

Root biomass increased with time and litter distribution effects were only detectable at the final root biomass sampling (Figure 4.7). After physiological maturity, the root biomass in response to uniformly distributed litter was less than the mean root response to the aggregated litter distributions (P<0.006). The R:S

was similar at each sampling date in 2006 with a mean of 0.54 at DOY 188, 0.28 at DOY 226, and 0.18 at DOY 269.

Soil moisture varied throughout the second experiment in the surface 190 mm from 0.04 to 15.2 g H_2O g $soil^{-1}$ and in the bottom of the containers from 0.03 to 18.8 g H_2O g $soil^{-1}$on DOY 234 and 285, respectively, and was unaffected by soil treatment ($P>0.05$).

Root induced soil surface CO_2 flux

The soil surface CO_2 flux was on average greater from planted containers treated with a uniform litter distribution than from containers with an aggregated litter distribution ($P<0.004$; Table 4.5). The contributions to the ANOVA variance from individual sampling dates were fairly consistent across the season with F-test values ranging only from 0.25 to 2.96, indicating that the difference in CO_2 flux between the litter amendments was relatively consistent (soil amendment x date: $P<0.402$; Table 4.5). Soil surface CO_2 flux from containers without maize plants was on average 3.7 mg C m^{-2} h^{-1} greater from soil amended with litter than from the control soil treatment ($P>0.047$; Figure 4.8). Interpolated across the entire sampling period (DOY 160 to 233), this represents a difference of 0.97 g C $container^{-1}$ or 6.3% of the total litter C added.

The difference in soil surface CO_2 between planted and unplanted containers is an indication of the plant effect on C cycling in this model system. The interpolated average difference between the planted and unplanted control +N was 41 mg C m^{-2} h^{-1}, whereas the difference in soil surface CO_2 flux between litter amended containers was greatest in response to the uniform distribution (73

mg C m^{-2} h^{-1}) and lower in response to 8, 24, and 72 patches (44, 43, 40 mg C m^{-2} h^{-1}, respectively). Most of the differences between soil surface CO_2 flux of planted and unplanted containers occurred during the last four sampling dates (DOY 200, 208, 217, and 233)(Figure 4.8) when 76% of the CO_2 flux on average was attributable to the plant effect.

DISCUSSION

Our results demonstrate that spatial heterogeneity of labile organic N resources within the reach of individual maize plants can influence above and belowground productivity of field grown maize; however, the plant growth response to resource heterogeneity was dependent on the resource substrate (litter species). Plant productivity has been found to be enhanced, suppressed, and remain unaffected by soil resource spatial heterogeneity (Hutchings et al. 2003). Experiments manipulating resource heterogeneity in single and multiple plant systems suggest the characteristics of both the resource and the plant roots and root associates can contribute to the idiosyncratic productivity responses (Fitter et al. 2000).

Resource Properties

The form, duration, spatial distribution, size, and intensity of soil resource patches versus a uniform distribution of that resource all potentially contribute plant productivity responses to resource heterogeneity (Fitter et al. 2000). In the research presented here we aggregated plant litter, a biochemically heterogeneous substrate, into patches surrounded by soil. The litter aggregates ranged in size from 0.52 (Experiment #2) to 7.2 g of clover litter (Experiment #1)

and up to 9.0 g of rye litter (Experiment #1). Across this range in patch sizes we did not observe any changes maize productivity. The only other study to distribute plant litter into multiple patches (1 or 4 patches) also found no differences across patch sizes (Bonkowski et al. 2000).

It is interesting to consider the scale of the resource patches relative to the organisms involved in this plant-soil system. We estimate that each of finely-ground red clover particles is about 10 µg. Thus if we consider the uniform distribution in our study as about 4 million very small (10 µg) patches, than there are about 55,000 times more patches in the uniform distribution than in the 72 patch treatment. This is potentially a functionally significant jump in patch size and number for plant productivity and microbial habitat. On the microbial scale a 10 or 100 µg patch of substrate is quite large; however, on the plant root scale patches in this size range may be indistinguishable from the background soil. In our previous work on the influence patch size on litter decomposition of this same soil-clover litter combination, we observed a 3 to 5 d lag in the maximum decomposition rate when litter is aggregated relative to uniformly distributed (Chapter 3). This indicates that the patch size had at least a transient effect on microbial processes, although, it is unclear if this microbial response is linked to the plant growth response.

Although we cannot directly test for the effect of the spatial distribution of the litter aggregates into clustered or random distributions, the similar maize response in both experiments indicates that this is was not a significant factor.

The duration a resource patch remains distinguishable from the background soil by a plant root is likely a product of the dispersal and consumption rate of the resource (Fitter et al. 2000). The dispersal rate of a resource is in turn controlled by its solubility in the soil solution, movement of the soil solution, and diffusion rate. Plant litter-N is mostly contained in insoluble organic compounds and thus to be assimilated by plants must be mineralized into more soluble forms such as NH_4^+, NO_3^-, and perhaps amino acids. The N mineralization rate from plant litter in a given soil environment is largely dependent on the N, C, phenolic, and lignin content of the litter. Measureable N mineralization can continue for months to decades depending on plant litter characteristics (Parton et al. 2007). For the relatively N-rich litters that we used in these experiments, N mineralization during the first year following soil application likely varied from 30 to 60% of the initial litter-N (Harris et al. 1994, Honeycutt 1999). Thus even at the end of the first growing season about half of the original litter-N should remain in the litter (Chapter 5).

Plant root proliferation into patches of limiting resources varies with patch composition (Hodge 2006). In a review of plant root responses to resource patches, Robinson (1996) asked why do plants bother proliferating roots into patches of highly mobile ions such as NO_3^- given that often times the NO_3^- is depleted before a root biomass proliferation is evoked. Furthermore, he pointed out that roots proliferate to a similar degree in patches of nitrate and phosphate ions (Drew 1975), despite their differences in soil mobility. Phosphate ions are typically at too low a concentration in the soil solution for plants to meet their P

needs by bulk flow alone (Kovar and Barber 1989) and thus diffusion of P ions is required to meet plant P demands. Hence proliferating roots into patches of higher than background phosphate ions is advantageous because it limits the distance that phosphate ions have to diffuse to be taken up by the plant root. Likewise, plant root proliferation in response to patches of NO_3^- and NH_4^+ appears more advantageous if the resource patch has limited solubility and considerable duration as is the case if the inorganic N is derived from a patch of mineralizing organic matter (Hodge et al. 1999). Furthermore, root proliferation into a patch of organic matter under N limited conditions potentially alters plant-plant (Hodge 2006) and plant-microbe N (Wang and Bakken 1997) competition because the distribution of N-limited plants and soil microbes are also likely heterogeneously distributed. Thus intercepting patch derived inorganic N ions before other plants or N-limited microbes improves plant fitness under either wise generally N-limited conditions.

We are unaware of other studies that have used more than one type of complex organic N (e.g., plant litter) as the heterogeneously distributed resource, so we have no comparison to draw on to explain why the two litter species used in Experiment #1 induced different responses from maize plants.

Aboveground and Belowground Biomass and C Allocation

Root to shoot biomass ratio (R:S) was unaffected by litter distribution during Experiment #2; however, there was a significant alteration of the soil surface CO_2 flux in response to the litter distribution that is likely associated with root C allocation. Optimization principles suggest that R:S should vary to

allocate resource acquiring structures (roots and leaves) to match the distribution of growth limiting resources. However, resource heterogeneity within the scale of individual plants may alter plant resource acquisition efficiencies (Hutchings and John 2004). Several studies have indicated that under heterogeneous resource conditions (soil or light) plants will maximize resource capture from sites rich in resources other than from resource-scarce sites (see review by Hutchings and John 2004). This leads to greater resource acquisition efficiencies because fewer plant resources are required to acquire nutrients from concentrated resources than from diffuse resources (Kovar and Barber 1989). In contrast, root detection of resource pools in a spatially and temporally heterogeneous environment is likely complex and imperfect. Therefore morphological responses (e.g., root proliferation) to fleeting resource pools may have a negative impact on resource acquisition efficiencies because the costs may out weigh the benefit (Robinson 1996).

As soil resources acquisition structures, plant roots have both physiological and morphological responses to most soil nutrients. Physiological responses to resource availability are thought to precede any changes in morphological root structure. We saw no differences in morphological allocation of plant biomass among litter distributions; however, the soil surface CO_2 flux differences may be partly explained by changes in physiological processes occurring in the roots (Figure 4.8). More work has been conducted on physiological responses to soil NO_3^- than to NH_4^+ or amino acids so we will only speculate on the role of NO_3^- here. In general, physiological root responses to

low soil NO_3^- concentrations include the induction of high affinity transport systems specific for NO_3^- and NO_3^- reduction in roots (Tischner 2000). Could the up regulation of these or other physiological processes partly explain the differences in CO_2 soil surface flux among the litter distribution treatments? Further work needs to be conducted in this area because if the uniform litter distribution induces increased root respiration than this can have major implications for interpreting CO_2 flux in many ecosystems.

In contrast, the differences in soil surface CO_2 fluxes among the litter distribution treatment of Experiment #2 may be attributed to greater rhizosphere induced oxidation of the litter or soil organic matter, the so called priming effect. Cheng et al. (2003) found that rhizosphere priming was sensitive to plant phenology and species; however, it was not sensitive to fertilization. Although many hypotheses have been put forward to explain the priming effect there is still little actually known of its importance to ecosystem level processes (Kuzyakov 2002).

The heterogeneity of agronomically managed soils is well characterized at scales ranging from 1m to 1 km scales (Robertson et al. 1993); however, less attention has been paid to spatial scales within the influence of individual crop plants (Franklin and Mills 2003, Han et al. 2007). Maize plants are known to selectively forage for patches of soil P (Zhu et al. 2005) and are potentially more sensitive to the spatial distribution of P fertilizer than to the quantity of P applied (Kume et al. 2006). Under N limited conditions we have shown that maize productivity is sometimes positively affected by the aggregation of plant litter

against an otherwise homogeneous soil background. Although it is unclear how the heterogeneity of agricultural soils compares to our experimental conditions, it is likely that management operations alter the distribution of crop and cover crop litters in the range that we manipulated the litter distribution. The duration of the litter patches as N-rich patches is likely a key component.

CONCLUSIONS

Aggregated red clover litter led to a 14% greater production of maize aboveground biomass relative to uniformly distributed litter regardless of whether the litter was clustered into 23% of the soil or randomly distributed throughout 88% of the soil. In contrast, the distribution of rye litter had no effect on maize growth. Litter distribution did not affect the biomass root to shoot ratio, but belowground C allocation to roots was substantially greater in the uniform litter distribution than in the aggregated distribution. The changes in belowground C allocation appeared to precede changes in the aboveground and belowground biomass and thus may provide a mechanistic link between plant response to resource heterogeneity and the functional significance of heterogeneity on ecosystem processes.

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Table 4.1 Repeated measures analysis of variance to determine effects of clustered soil amendments on aboveground maize biomass during Experiment #1.

Source	Num. df†	Den. df†	F value	P>F
Block	3	68	1.31	0.2796
Soil Amendment	5	68	7.65	<0.0001
Sampling Date	3	68	1187	<0.0001
Soil Amend. x Date	15	68	3.82	<0.0001

† Num df and Den df denote the numerator and denominator degrees of freedom used in F tests, respectively.

Table 4.2 Analysis of variance to determine effects of soil amendments on aboveground maize biomass for each sampling date during Experiment #1. Contrasts compare the mean effect of aggregated litter versus uniform litter distribution between (interaction) and among the two litter species.

			Sampling Date (day of year)							
			-------201-------		-------215-------		------235‡------		-------279-------	
Source	Num. df†	Den. df†	F	P>F	F	P>F	F	P>F	F	P>F
Block	3	15	1.32	0.305	0.62	0.610	0.71	0.563	1.38	0.288
Soil Amendment	5	15	2.15	0.115	1.78	0.178	1.82	0.173	8.50	<0.001
Contrasts – Litter Dist.										
Clover litter Dist.	1	15	0.00	0.955	0.00	0.968	3.69	0.075	5.87	0.029
Rye litter Dist.	1	15	0.65	0.434	0.00	0.952	0.09	0.769	0.50	0.489
Litter species x Dist.	1	15	0.12	0.730	0.00	0.996	3.32	0.090	6.02	0.027

† Num df and Den df denote the numerator and denominator degrees of freedom used in F tests, respectively.
‡ the denominator degrees of freedom for sampling day of year (DOY) 235 is 14, one less than the other DOYs.

Table 4.3 Repeated measures analysis of variance to determine effects of random litter distribution on aboveground maize biomass during Experiment #2.

Source	Num. df†	Den. df†	F value	P>F
Block	3	81	0.30	0.822
Litter Distribution	4	81	3.76	0.008
Sampling Date	5	81	825	<0.001
Litter Dist. x Date	20	81	1.45	0.126
Contrast				
Uniform vs. Aggregated	1	81	5.22	0.025
Uniform vs. +N control	1	81	11.27	0.001
Aggregated vs. +N control	1	81	3.61	0.061

† Num df and Den df denote the numerator and denominator degrees of freedom used in F tests, respectively.

Table 4.4 Partition of the variance attributable to litter distribution by sampling date (DOY) interaction effect of a repeated measures analysis of variance to determine effects of random litter distribution on aboveground maize biomass during Experiment #2.

Source	DOY	Num. df†	Den. df†	F value	P>F
Litter dist. x DOY	188	4	81	0.41	0.801
	201	4	81	0.40	0.811
	226	4	81	0.40	0.806
	234	4	81	0.21	0.932
	269	4	81	3.43	0.012
	285	4	81	2.73	0.035

† Num df and Den df denote the numerator and denominator degrees of freedom used in F tests, respectively.

Table 4.5 Repeated measures analysis of variance to determine effects of soil amendments of randomly distributed litter aggregates and +N control treatments on soil surface CO_2 flux from containers with maize plants during Experiment #2.

Source	Num. df†	Den. df†	F value	P>F
Block	3	110	2.35	0.0761
Soil Amendment	4	110	3.42	0.0113
Sampling Date	7	110	75.4	<0.0001
Amendment x Date	28	110	1.06	0.4016
Contrast – Litter Distribution				
Uniform vs. Aggregated	1	110	8.76	0.0038

† Num df and Den df denote the numerator and denominator degrees of freedom used in F tests, respectively.

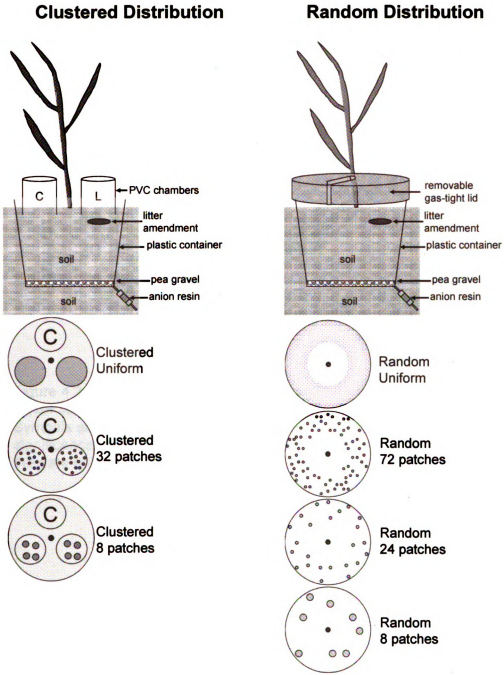

Figure 4.1 Schematic depiction of the spatial layout of litter distribution treatment for the clustered (Experiment #1) and random (Experiment #2) distribution experiments conducted in 50-L plastic containers set into pits in the soil and surrounded by other maize plants. In the top panels are side-cut views of the container components and vertical layout. At the bottom top-down views illustrating the spatial distribution of the litter treatments.

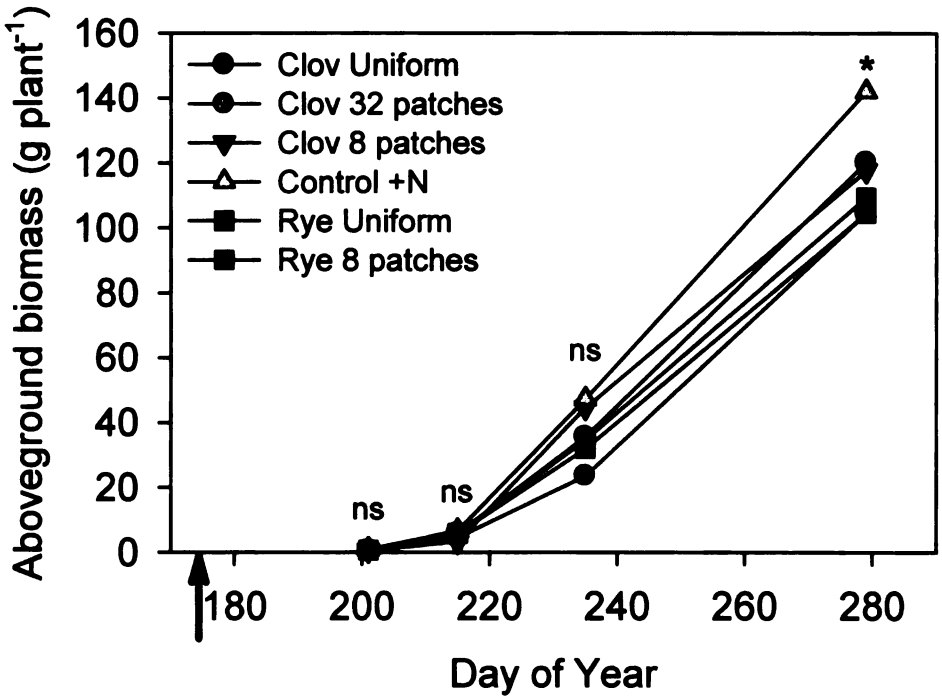

Figure 4.2 Aboveground biomass response to the distribution of rye and red clover litter and +N control soil amendments during Experiment #1. Symbols above data from each sampling date represents statistical significance, ns = $P>0.05$ and * = $P<0.05$. The arrow on the x-axis indicates the date of litter application to soil in the field containers.

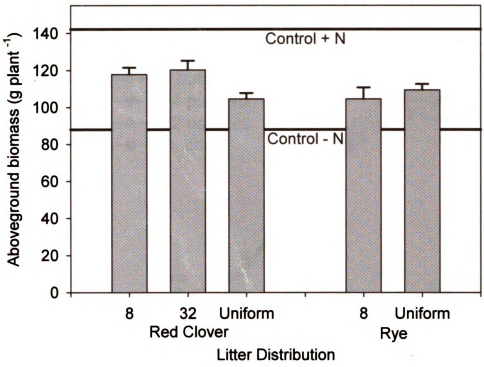

Figure 4.3 Final harvest aboveground biomass response to red clover and rye litter distributed into 8, or 32 patches or uniformly and 8 patches or uniformly, respectively, during Experiment #1. Vertical bars represent treatment means ± 1 standard error of the mean. The two horizontal lines represent aboveground biomass in response to containers fertilized with 1.6 g N container^{-1} as KNO_3 (control + N) or not fertilized (control – N).

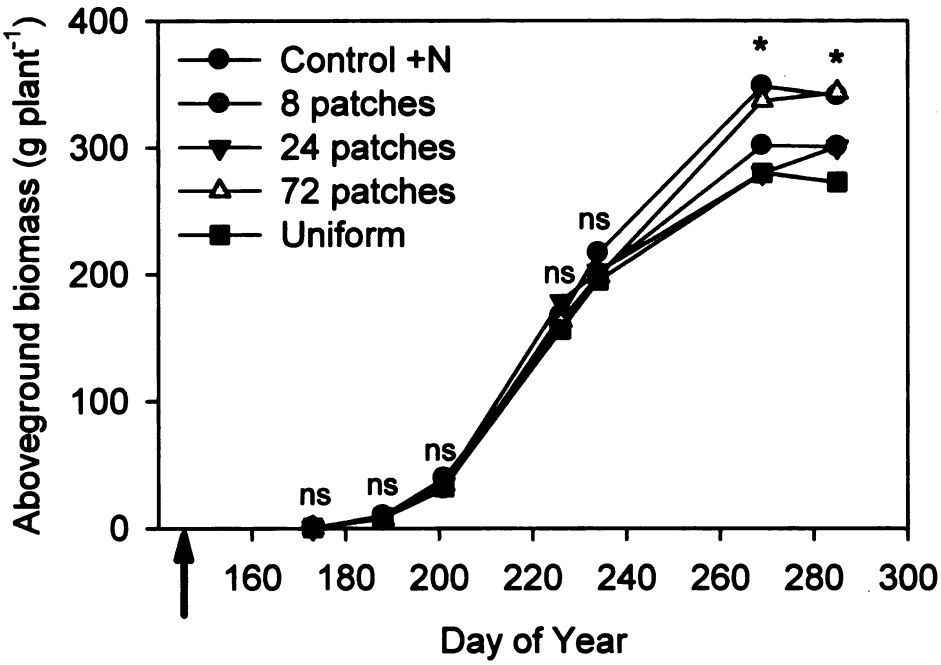

Figure 4.4 Aboveground biomass response to red clover litter distributed into 8, 24, or 72 patches or uniformly and +N control soil amendments during Experiment #2. Symbols above data from each sampling date represents statistical significance, ns = $P>0.05$ and * = $P<0.05$. The arrow on the x-axis indicates the date of litter application to soil in the field containers.

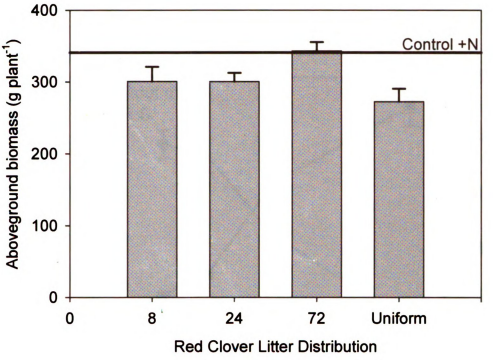

Figure 4.5 Final harvest aboveground biomass response to red clover litter distributed into 8, 24, or 72 patches or uniformly from Experiment #2. Vertical bars represent treatment means ± 1 standard error of the mean. The horizontal line represents final aboveground biomass in response to containers fertilized with 1.6 g N container^{-1} as $NaNO_3$ (control + N).

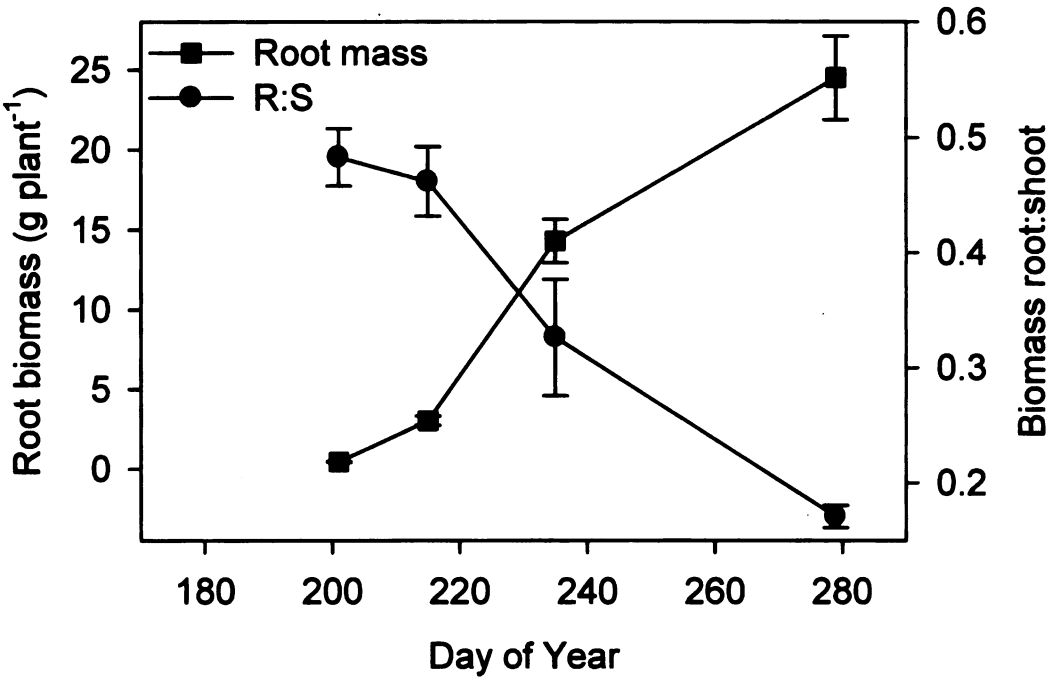

Figure 4.6 Root biomass and root to shoot ratio response to control +N treatment during Experiment #1.

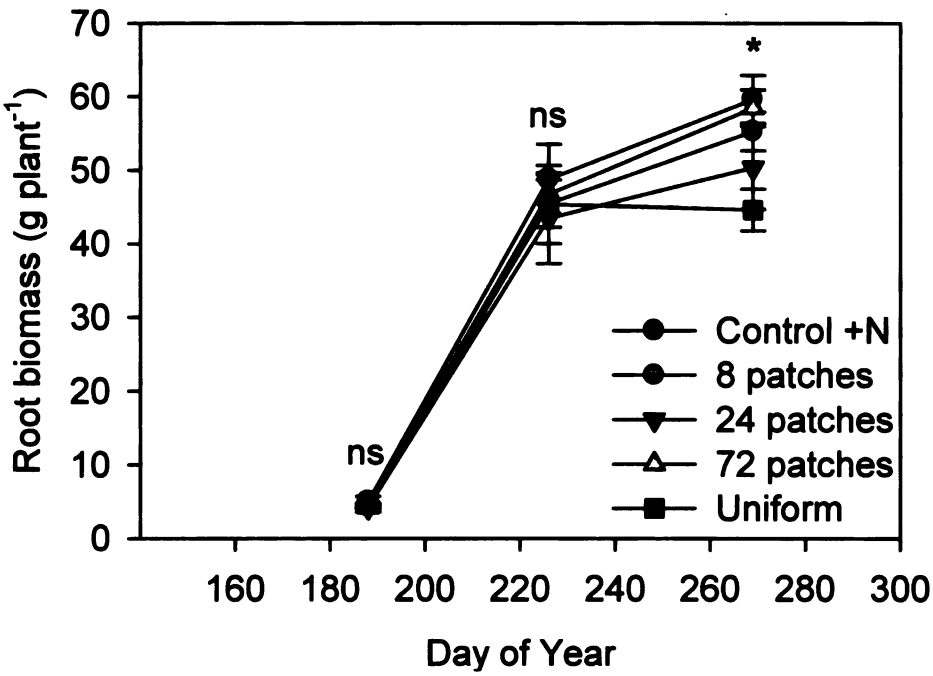

Figure 4.7 Root biomass response to red clover litter distributed into 8, 24, or 72 patches or uniformly and +N control soil amendments during Experiment #2. Symbols above data from each sampling date represents statistical significance, ns = P>0.05 and * = P<0.05.

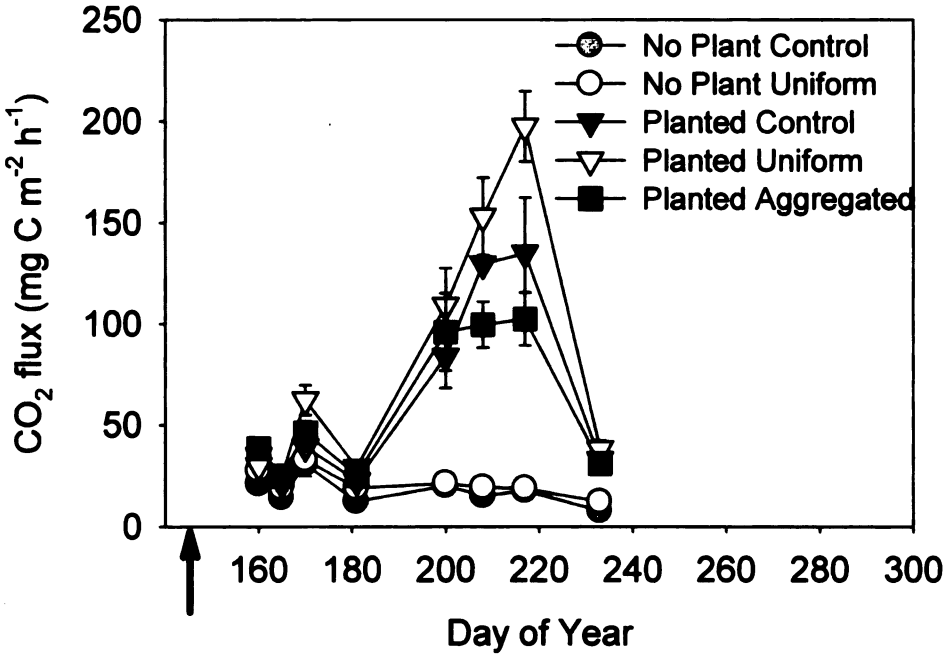

Figure 4.8 Soil surface CO_2 flux with and without maize plants and with no litter, uniform litter, or aggregated litter (8, 24, and 72 patches) during Experiment #2. Error bars denote one standard error of the mean CO_2 flux. The repeated measures ANOVA of the containers with growing plants indicated no significant litter distribution x sampling date interaction and a significantly greater CO_2 flux from uniform vs. aggregated litter. The arrow on the x-axis denotes the time of container deployment to the field.

Chapter 5: Litter Aggregation Alters Terrestrial Nitrogen Cycling

INTRODUCTION

Nitrogen mineralized from plant litter is the primary source of plant available N in both managed and unmanaged ecosystems. The spatial aggregation of plant litter in soil can potentially alter terrestrial N cycling by several mechanisms. The mineralization of litter-N into soluble N compounds and inorganic ions can be stimulated (Breland 1994) or inhibited (Magid et al. 2006) by its aggregation. Physical and chemical interactions between plant litter and soil mineral surfaces or soil organic matter can decrease soil microbial access to the litter and inhibited its decomposition. In contrast, aggregated litter has less physical contact with soil and hence potentially less protection from soil microbial degradation (Breland 1994). Alternatively, the diffusion of resources limiting heterotrophic activity (e.g., O_2 or NO_3^-) is more likely to inhibit decomposition and N mineralization in aggregated litter than uniformly distributed litter (Myrold and Tiedje 1985, Magid et al. 2006).

As litter-N is mineralized to NH_4^+ its fate can be altered by the distributions of: 1) nitrifier communities surrounding the litter aggregate (Nielsen and Revsbech 1998), 2) plant roots proliferating into the litter aggregates (Wang and Bakken 1997), and 3) proximal N-poor microsites where NH_4^+ may be assimilated into microbial biomass (Schimel and Bennett 2004). In most soils, NO_3^- is more mobile than NH_4^+ and can be readily transported with hydrologic flow to plant roots or below the plant rooting zone. Nitrate can also be used as an electron acceptor during denitrification under anoxic soil conditions, which are

more likely to occur when labile organic matter is aggregated rather than uniformly distributed due to diffusional constraints of O_2 (Parkin 1987).

Plant root systems have plastic morphological and physiological responses allowing for selective foraging into resource rich microsites (Drew 1975). However, some plants only benefit nutritionally from proliferating roots into microsites of complex organic matter and not from microsites of inorganic nutrients (vanVuuren et al. 1996, Hodge et al. 1999), despite the fact that plants primarily meet their nutritional demands by acquiring inorganic nutrients from soil. Furthermore plant roots modify soil conditions by consuming O_2, water, and inorganic nutrients while exuding reduced C compounds and stimulate C and N mineralization from soils (Cheng et al. 2003, Herman et al. 2006). As roots forage for heterogeneously distributed soil resources they potentially modify C and N cycling processes in resource-rich microsites more than in resource-poor microsites.

Our previous research indicates that plant litter aggregation has a minor influence on litter decomposition, thus we predict that the coupled process of litter-N mineralization will also be minimally affected. However, the interactions between root foraging and microbial responses to heterogeneous resource distributions may alter ecosystem level N cycling in ways not predicted from microbial responses alone. In this chapter, we distributed ^{15}N-labeled plant litter across an aggregation gradient in soil with growing plants to address these questions: 1) does resource aggregation influence above and belowground plant

N acquisition; 2) does resource aggregation influence net N mineralization; and 3) does resource aggregation influence whole system N retention?

MATERIALS AND METHODS

We conducted this study at the W.K. Kellogg Biological Station (KBS) in Southwest Michigan (42° 24'N, 85°24'W, elevation 288 m). We used a 1:1 mix of coarse sand and a composite of soil taken from the surface 0.4 m soil of a field on the KBS Long-Term Ecological Research site in this experiment. Kalamazoo (fine-loamy, mixed, mesic Typic Hapludalfs) and Oshtemo (coarse-loamy, mixed mesic Typic Hapluadalfs) soil series co-occur on the site and are both present in the soil used in this experiment. We excavated soil from the field, mixed with an end-loader, placed in a tarp covered pile to air dry, and after 12 months we power sieved the soil through a 12 mm screen, combined with the sand in a 250-L mixer, and stored in a common covered pile until use.

We used a Randomized Complete Block Design with 5 soil amendment treatments with plants and two amendment treatments without plants, 7 sampling dates, and 4 replicates per block. The 5 amendment treatments consisted of four litter distributions (8, 24, and 72 patches and uniform) and control +N fertilizer treatment. Each experimental unit consisted of a 50-L black plastic container filled with the soil mix, amendment, and a single maize plant (Pioneer ® 35Y54) (Fig. 1). We used *Trifolium pratense* (red clover) (variety Michigan Medium Red, Michigan State Seed Solutions, Grand Ledge, MI) shoots as the plant litter amendments.

We produced the clover in sand in a greenhouse, fertilized it with a modified complete Hoagland's nutrient solution containing ~ 8 atom % ^{15}N-KNO_3 as the N source (Hewitt 1966), and harvested it before initiation of the reproductive phase. The shoots were cut at the sand surface, dried for 4-5 days at 55°C, coarsely-chopped to pass a 10 mm screen with a Wiley® mill, and then finely-ground to pass a 1 mm screen in a Cyclotec® 1093 sample mill. Eight subsamples of finely-ground litter were analyzed for C and N content with a Costech ECS 4010 CHN elemental analyzer to determine application rate. The red clover litter used in this experiment contained 413 g C kg litter^{-1} and 31.0 g N kg litter^{-1} (5.81 atom% ^{15}N) for a C:N ratio of 13.3.

Field containers

The 50-L (370 mm in depth, 390 mm bottom diameter and 440 mm top diameter) containers were prepared by cutting 13 mm drainage hole in the bottom of the containers, adding 3 L of 8 to 10 mm diameter gravel to the bottom of the container to aid drainage, adding 40 L of soil mix to the container, adding the soil amendment, and 10 L more of soil mix, and then placing the containers into the holes in the field that were created when the soil was originally excavated. We placed the containers in the field flush with the soil surface and at least 2.5 m from one another. Containers have a surface area of about 0.15 m^2, which is similar to the standard production practices in this region of 0.14 m^2 plant^{-1}. We planted the rows between the containers with the same maize hybrid.

The containers to be harvested on the third and final sample dates were outfitted with anion exchange resin columns to capture NO_3^- draining out of the containers. Anion exchange columns consisted of 30 mL of Type II 16-50 mesh anion exchange resin (IONAC® A-554 Cl-form) in a 250 mm length of 32 mm diameter clear vinyl tubing with 8 µm fiberglass on each end (Corning Inc., Corning, NY). Preliminary analysis showed that this column design had an anion exchange capacity in excess of 250 mg $N\text{-}NO_3^-$ in a soil solution matrix (unpublished data) and that NO_3^- held on the exchange resin was stable (no detectable denitrification losses) under 4°C and 22°C conditions for at least 60 days. The columns were fitted to the outside of each container's drain hole with vinyl fittings and rubber gaskets and sealed with 100% silicon rubber sealant (DAP Inc., Baltimore, MD). The opening of the fitting into the container was covered with a garden hole screen (~0.5 mm) to keep sediment from flowing into the column, and the containers were tilted about 1% towards the drain holes to aid drainage.

Litter application

The finely-ground clover litter was distributed into each container into one of 4 configurations: eight 4.69g patches, twenty 1.56 g patches, seventy-two 0.52 g patches, or a uniformly distributed 37.5 g patch. To avoid disturbing the patches when planting the maize litter was kept from center 150 mm of each container (Figure 5.1). The litter distribution treatments were constructed by placing a temporary circular template of the same diameter as the inside of the 50-L container on top of the initial 40 L of soil-mix, adding the litter to the

template, removing the template, and then adding 10 L more soil mix on top of the litter to a mean depth of 100 mm. Litter application took three days from May 22-24, 2006 during which the containers were protected from precipitation.

The template was a 440 mm diameter circular sheet of acrylic with eight 21.3 mm, twenty-four 12.1 mm, or seventy-two 8.3 mm diameter plastic syringe barrels adhered perpendicular to the sheet. The "needle end" of the syringe barrels and the rubber tips of the plungers were removed. We used the syringe plungers to displace a consistent volume of soil within the syringe cylinders before adding the litter. We pushed the plungers down into the cylinders as we raised the template such that the litter remained in the soil as individual column shaped patches just below the surface of the soil. After the template was removed we added 10 L of soil on top of the litter. Each patch of litter had the same mean depth in the soil. The uniform treatment was constructed by mixing 37.5 g of litter with 4 L of soil taken from to the containers, then adding to the litter-soil mix back into the outer ring of the soil in the container (Figure 5.1).

Planting and fertilizing

On May 25, 2006, the containers were transferred to the field and on May 26 the anion exchange columns were installed. We place two maize seeds (Pioneer 35Y54) to a depth of ~50 mm into the center of each container on May 31 and covered with hardware cloth exclosures to prevent rodent damage. Maize seeds were also planted between the containers at standard production densities (70,000 plants ha^{-1}). After 14 days the seedlings were thinned to one plant $container^{-1}$ and the exclosures were removed.

We designed this experiment to test maize response to litter distribution in a system where N is the only soil nutrient limiting plant growth. On July 6, 2006, 250 mL of nutrient solution containing 0.5 P g, 1.25 K g, 0.25 S g, 0.27 Ca g, and 0.07 Mg g was added to the soil surface of each container. The control +N treatment was also implemented on July 6, 2006 by adding 1 L of solution containing 1.6 g N as NaNO3 to each container of the control +N treatment, whereas the control -N and litter treatments all received 1 L of RO (reverse osmosis) water. Supplemental watering (2 L container^{-1}) occurred on August 13 and 22.

Repeated harvest

We conducted seven repeated whole container harvests. Four of the harvests (June 22, July 20, August 22, and October 12, 2006) were made to quantify maize shoot N content and soil N pools and three others (July 7, August 14, and September 26, 2006) to quantify maize shoot and root N content. The aboveground shoots were cut at the soil surface, dried for 4 days at 60°C, weighed, ground to pass a 1-mm screen, and analyzed for total N and atom% ^{15}N by mass spectrometry at the U of California - Davis Stable Isotope Facility. We transported the containers to a laboratory for processing to determine soil moisture and total N contents in the surface 190 mm and the bottom 180 mm of each container. Inorganic N also was determined in the surface 190 mm of soil. The soil and roots were sieved to pass a 6 mm screen, homogenized, weighed, and three sub-samples were dried for 3 days at 65°C and reweighed. A sub-

sample of the dried soil was then pulverized with a roller mill and analyzed for N and ^{15}N content.

Three 20 g fresh soil sub-samples of the homogenized soil were extracted from the surface soil of each container with 100 mL of 2M KCl, shaken, allowed to equilibrate for 24 h, shaken again, allowed to settle for 1 h and then filtered (Type A/E Glass Fiber Filter, Pall Life Sciences, Ann Arbor, MI, USA). Filtered extracts were frozen until analyzed for NH_4^+ and NO_3^- on an Alphem Flow Injection Analyzer (NH_4^+ via diffusion colorimetry technique and NO_3^- via cadmium reduction and colorimetry) (OI Analytical, Collage Station, TX, USA). The inorganic N contained in the KCl extracts were reduced to NH_3 (Devarda's alloy) and diffused onto acidified (H_2SO_4) 10 mm diameter glass fiber filter paper during a 14 d incubation at 40°C with MgO to obtain a solid sample to analyze for ^{15}N content (as above) (Burke et al. 1990).

We followed the fate of the litter-derived N throughout the season into maize roots and shoots, soil inorganic N, leached NO_3^-, total soil N pools by ^{15}N tracer methodologies (Powlson and Barraclough 1993). Atom % ^{15}N excess is equivalent atom % ^{15}N minus 0.3663. The percentage of litter -^{15}N recovered in each N pool was calculated as described in Hauck (1982) and Jackson (2000) using the equation:

Percentage recovery of litter-15N = ([{N(Ao/100)} – {N(Ai/100)}]/15NL)*100

where N is mg$^{14+15}N$ container^{-1} of a sampled pool (inorganic N, total soil N, shoot N, root N, or leached N), A_o is the atom % ^{15}N excess of a sampled pool, A_i is the atom % ^{15}N excess of the initial pool in the control treatment, and $^{15}N_L$ is

mg litter-^{15}N container^{-1} added to the soil. The portion of each N pool that was derived from the litter-N was calculated as:

Portion derived from litter-N = $\{N(A_o - A_i)\}/A_L$

where A_L is the atom % ^{15}N excess of the litter added to the soil. The mg litter-derived N container^{-1} was the product of the portion derived from litter-N and the mg N container^{-1} of each pool.

Because we did not sample root and soil N pools on the same harvest dates we estimated the root biomass on DOY 173, 201, 234, and 285 by linear interpolation of the root to shoot ratio from the root harvest dates. We also estimated the root ^{15}N and N concentration in same manner. The product of the root biomass and the ^{15}N and N concentration were then used to estimate the root N and ^{15}N content on DOY 173, 201, 234, and 285. The mineralization of litter-derived N was estimated as the sum of the litter-derived N in the shoot, root, soil inorganic N, and leached N pools on DOY 173, 201, 234, and 285.

Statistical Analysis

A repeated measure analysis of variance (ANOVA) was performed for each response variable with block and soil amendment treatment as independent fixed variables with sampling date the repeated random variable. We used Akaike's information criteria (Akaike 1974) to choose a first-order heterogeneous autoregressive covariance structure to model the repeated measure variance components for all of the response variables except the litter-derived root and leachate N. All statistical tests were conducted using SAS mixed model procedures (Littell et al. 2005). Natural log transformations of the data were

performed prior to analysis when required to meet the assumption of ANOVA. Differences were considered significant at the $\alpha < 0.05$ level for all ANOVAs.

RESULTS

Soil Inorganic N and Litter-derived Soil Inorganic N

The extractable inorganic N in the surface 190 mm of soil in response to the litter distribution varied with sampling date throughout the growing season (distribution x date; P<0.0001, Table 5.1 and Figure 5.2). The soil inorganic N and the proportion of this inorganic N that was derived from the litter-N generally decreased as the season progressed (Figure 5.2). By the first sampling date (28 days after soil application of red clover litter) the soil inorganic N was increased in response to all of the litter distribution treatments to a similar degree (P<0.68, averaging 440 mg N container^{-1} or 20.5 μg g soil^{-1}) relative to the yet unfertilized control (Table 5.2 and Figure 5.2). After N fertilization on DOY 201, the soil inorganic N pool of the control +N treatment was higher than the litter amended soils. On DOY 201 and 285, the soil amended with aggregated litter (8, 24, and 72 patches) averaged 54% (P<0.032) and 15% (P<0.007) less soil inorganic N, respectively, than soils treated with a uniform litter distribution (Table 5.2). The litter-derived soil inorganic N in response to litter distribution varied with sampling date early in the season (distribution x date; P<0.0001, Table 5.3) and was not determined during the last two sampling dates due to the small pool sizes (Figure 5.2).

Plant N and litter-derived N content

Maize shoot N varied in response to litter distribution treatments throughout the season (distribution x date; $P<0.007$, Table 5.5 and Figure 5.3) and did not differ on any individual sampling date (Table 5.6). By the end of the season maize plants on average accumulated 2.8 g N shoot^{-1} with litter amendments and 3.3 g N shoot^{-1} with the control +N fertilization. The plants grown in soils amended with litter aggregated into 8, 24, and 72 patches on average acquired a greater proportion (18%, $P<0.0001$) of their shoot N from the litter than did plants grown in soils amended with a uniform litter distribution (Figure 5.3 and Table 5.7).

Maize root N response to litter distribution also varied throughout the growing season (distribution x date; $P<0.01$, Table 5.8 and Figure 5.4). At the end of the growing season (DOY 269) root N accumulation was 27% greater in the aggregated litter distributions (8, 24, and 72 patches) (0.38 g N container^{-1}) than in the uniformly distributed litter treated soils (0.30 g N container^{-1})($P<0.016$, Table 5.9 and Figure 5.4). The proportion of the litter-derived N in the roots was 33% greater on average in plants grown in soils amended with aggregated litter (8, 24, and 72 patches) than the uniform litter distribution ($P<0.0001$, Table 5.10 and Figure 5.4).

Leachate and litter-derived nitrate-N

The nitrate-N leached from the containers was low regardless of how the litter was distributed in the soil or when sampling occurred, averaging 21 mg N container^{-1} across all treatments and both sampling dates (Table 5.11 and Figure 5.5). The proportion of the NO_3^- leached that was litter derived, however, was

affected by the time of sampling such that a greater proportion of the NO_3^- leached during the entire season (DOY 285) than after 56 days in the soil (DOY 201) (date; P<0.016, Table 5.12).

Accumulated litter-derived mineralized N

The mineralized litter-derived N from litter distribution varied throughout the season and accumulated from an average of 175 on DOY 173 to 379 mg litter-N container^{-1} on DOY 285 (distribution x date; P<0.008, Table 5.13 and Figure 5.6). The aggregated litter distributions accumulated 28, 8, 21 and 24% more litter-derived mineralized N on DOY 173, 201, 234, and 285 (P<0.046, 0.218, 0.010, and 0.0003), respectively, than did soils amended with a uniform litter distribution (Table 5.14 and Figure 5.6).

Percent litter-^{15}N recovery

The majority (50 to 60%) of the litter-N remained in the soil, presumably in an organic form, at the end of the growing season (Table 5.15). No differences in this pool were observed among distribution treatments (P<0.14). The maize plants (shoots and roots) were the second most important sink for the litter-N, accounting for ~30% of the total litter-N added to the soil. At the final harvest litter-derived N in the maize tissue was 24% greater in response to the aggregate litter distributions (8, 24, and 72 patches) than the uniform litter distribution (P<0.0003). The total litter-derived NO_3^- leached during the season was less than 0.1% of the litter-N for each litter application treatment. Likewise, the soil inorganic N pool at the end of the growing season only contained 0.1 to 0.2% of

the litter-N added. The balance of the litter-N (the unaccounted for mass of litter ^{15}N) varied from 12 to 19% across the litter treatments at the end of the season.

DISCUSSION

Our results demonstrate that resource heterogeneity at spatial scales influenced by individual plant root systems can significantly alter N cycling rates. The spatial distribution of plant litter in soil had a statistically significant but minor influence on maize root N content and a dramatic but short-term effect on soil inorganic N in the surface soil layer containing the litter. In contrast, litter distribution had a major effect on the fate of litter-derived N. Plants grown in soils with red clover litter distributed into aggregates obtained more of their tissue N from the litter despite only minor N limitations.

Why was litter-N cycling affected by litter aggregation?

The distribution may have influenced the litter-N fate by a number of possible mechanisms, both microbial and plant related. In the short term, following application of litter to soil there is a potential for litter aggregation to affect decomposition (Chapter 3), N mineralization, nitrification, N_2O emissions (Ambus et al. 2001, Chapter 3), and denitrification rates (Breland 1994, Nielsen and Revsbech 1998) occurring in and around the litter aggregates. The aggregation of plant litter has been shown to both stimulate (Breland 1994) and inhibit (Magid et al. 2006) decomposition rates relative to a uniform litter distribution. Our previous work incubating the same litter distribution-soil treatment combinations without plants revealed a 3 to 5 d delay in the maximum decomposition rate of the aggregated litter relative to the uniformly distributed

litter, but no longer-term changes in the decomposition rate (Chapter 3). Thus in the short-term (<15 days) it is unlikely that the soil-litter contact (Breland 1994) provided significant physical protection of the litter regardless of distribution. Likewise, microbial predation by nematodes and protozoa (Griffiths 1994) was unlikely to have responded quickly enough to litter distribution within the 3 to 5 d to act as a major controller of the litter decomposition in our system, though these mechanisms may be important later in the season. In contrast, litter distribution had a substantial effect on the N_2O emissions from litter amended soils without growing plants (Chapter 3), suggesting a significant alteration of N cycling in response to litter distribution.

Our data indicated that soil inorganic N pools liberated from the litter were substantially affected by the litter distribution suggesting that N cycling was dramatically different across the litter aggregation gradient (Figure 5.2). For example, 56 days after litter application (DOY 201), the average soil inorganic N content in response to aggregated litter (patches of 8, 24, and 72) was 54% less than the uniform litter distribution (151 vs. 329 mg N container^{-1}). This difference in soil inorganic N is likely due to both differences in processes producing (gross mineralization) and consuming (gross microbial immobilization, plant N uptake, denitrification, and hydrologic N losses) inorganic N.

Gross N mineralization rate, the transformation rate of organic N into inorganic N, is often assumed to be related to the heterotrophic respiration rate of a given substrate of known C:N (Luxhoi et al. 2006). We saw only a short-term influence of litter aggregation on respiration rates in the incubation study

(Chapter 3) and no detectable differences in the litter decomposition rate (Chapter 4), we thus have no indication that the gross N mineralization was influenced by the litter distribution. Plant accumulated N and leachate NO_3^- were similar at this point in the season (DOY 201) among litter distribution treatments and thus should not account for any difference in soil inorganic N. In both the laboratory incubations and this container experiment (Loecke Chapter 3), N_2O emission were considerably higher from the aggregated litter than the uniform litter; however, it is unclear how well N_2O emissions relate to total gaseous N losses (Mathieu et al. 2006).

The difference in soil inorganic N among the litter distribution treatments observed at DOY 201 occurred just prior to the most rapid plant grow rates of the season (Chapter 4) and thus had the potential to substantially affect maize growth and N acquisition (Blackmer et al. 1989). However, this early season plant available N pool and succeeding plant N accumulation were poorly correlated (Figures 5.2, 5.3, and 5.4). It was not until plant physiological maturity that the plant N content differed among the litter treatments. Surprisingly, this plant N difference was in the opposite direction as predicted from the soil inorganic N pool from earlier in the season, because soil inorganic N early in the season is often used as an indication of the plant available N for the entire season (Blackmer et al. 1989). Also at the end of the growing season, the soil inorganic N content in the surface 190 mm was 15% less in response to the aggregated treatments than the uniform litter distribution. This may indicate a

greater risk of post-harvest NO_3^- leaching from the uniform litter distribution as well as less complete utilization of available resources.

Maize root N content increased in response to aggregated litter, but shoot N content was unaffected by litter distribution. Other studies have found both negative (Hodge 2003) and positive (e.g., Bonkowski et al. 2000) effects of litter aggregation on the plant N uptake of individual plants. Several processes may explain why litter distribution altered plant N uptake and the proportion of litter-derived plant N. First, the plants may have only been weakly or not all N-limited at the point in the season when the differences in the soil inorganic N existed. Two observations support this supposition: 1) the root N content did not start to differentiate among litter treatments until after the soil inorganic N had dropped to insignificant levels after DOY 234; and 2) the control +N treatment only increased plant N content by 19% more than the average litter treatment. Alternatively, the pools of N accessed by the plant roots may have differed among the litter treatments, thus influencing the total plant N uptake and the litter-derived plant N. The proportion of litter-derived N in the maize roots and shoots was on average and especially early in the season was greater in response to the aggregated litter than the uniformly distributed litter. This implies that there was a greater spatial and temporal coupling of the plant N uptake and litter-N mineralization in the soils amended with aggregated litter than the uniform litter distribution.

Wang and Bakken (1997) hypothesized that the spatial coupling of litter-N mineralization and plant N uptake improves the synchrony of these two processes by more directly transferring the mineralized litter-N to plant roots

when the roots are spatially associated with the litter. This implies that root foraging for N-rich microsites can effectively alter plant-microbe competition for N if N-rich and N-poor microsites are sufficiently heterogeneously distributed at a scale at which plant roots can selectively proliferate into N-rich microsites and avoid N-poor microsites. Furthermore, rhizosphere induced N mineralization may have increased litter-N mineralization of the litter where the roots and litter spatially co-occur (Herman et al. 2006). Maize root systems are known to selectively proliferate into microsites rich in N (Chapter 2) and P (Kume et al. 2006); however, the scales at which maize roots perceive differences in microsite quality, intensity, duration, and size is unknown. We did not quantify root proliferation into the aggregated litter in this experiment because we wanted to preserve the ^{15}N balance of the system; however, we visually observed intense root proliferation into the litter aggregates.

Litter distribution had a substantial effect on litter-derived N mineralization in our study. Here we define litter-N mineralization as the transfer of litter-derived ^{15}N into plant tissue and into soil inorganic N, and leachate N pools. Litter-N mineralization was on average 20% greater in response to aggregated litter than to uniformly distributed litter. This is despite the observation that on two of the four dates measured, the pool of soil inorganic N was greater in response to the uniform distribution then the aggregated litter and were no different on the other two sampling dates. This may indicate a significant alteration of the extent that plants access N from labile organic matter pools. A 20% increase in litter-N mineralization is comparable to the effects of other basic alterations of soil

biological conditions. For example, the addition of microfauna grazing protozoans to denuded soil increased litter-N mineralization by a third (Bonkowski et al. 2000). From our previous work on the decomposition of aggregated litter, we predicted that the distribution of litter in soil would have only a minor influence on the mineralization of litter, thus either the plant and plant-microbe interaction or microbial dynamics that were not observable during the incubation played a significant role in altering N cycling in response to resource heterogeneity.

Fate of aggregated litter-N

At the end of the season most of the litter-N (as indicated by the ^{15}N label) was retained in the soil matrix (50 to 60%), presumably as organic N, regardless of the spatial distribution of the litter (Table 5.15). In contrast, the litter-derived plant N was affected by the litter distribution and accounted for about 30% of the litter-N added to the soil. This is a higher recovery of litter-N into maize tissue N than Harris et al. (1994) at 17% recovery of alfalfa litter N during the first season post application. Our higher recovery of litter-N in plant tissue may be because we used less fertile soil than Harris et al. Both leachate N and soil inorganic N at the end of the season were insignificant portions of the litter-N added. The unaccounted for litter-N was also not noticeably different among the litter distributions; however, it was a significant fate of the litter-N averaging 16% of the total litter-N applied. This missing N is likely due to denitrification losses. It is interesting to note that we were able to detect 6 to 7 fold differences in N_2O

emissions across the aggregation gradient (Chapter 3) but were not able to detect differences in total gaseous N losses.

CONCLUSIONS

Litter aggregation substantially altered N cycling in our system. The litter aggregation gradient had statistically significant but minor effects on total plant N content; however, plants growing in soil with aggregated litter derived 20% more of their N from the litter than plants grown in soils with a uniformly distributed litter. This difference in litter-derived plant-N is in contrast to the greater plant available inorganic N pools observed in response to the uniform distribution on two dates. Taken together these two observations suggest that a closer spatial coupling of roots and aggregated litter allowed for greater litter-N uptake while minimizing the risk of NO_3^- leaching.

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Table 5.1 Repeated measures analysis of variance to determine effects of litter distribution on soil inorganic N (NO_3^- and NH_4^+).

Source	Num. df†	Den. df†	F value	P>F
Block	3	50	23.98	<0.0001
Litter distribution	4	50	2.61	0.0467
Sampling Date	3	50	465.1	<0.0001
Distribution x Date	12	50	6.07	<0.0001

† Num df and Den df denote the numerator and denominator degrees of freedom used in F tests, respectively.

Table 5.2 Analysis of variance to determine effects of litter distribution on soil inorganic N (NO_3^- + NH_4^+) for each date sampled during the growing season. The contrasts compare the mean effect of aggregated litter versus uniform litter distribution.

			Sampling Date (day of year)							
			------173------		------201------		------234‡------		------285------	
Source	Num. df†	Den. df†	F	P>F	F	P>F	F	P>F	F	P>F
Block	3	10	0.26	0.853	0.50	0.691	3.10	0.071	25.2	0.001
Litter Distribution	4	10	11.7	0.001	6.52	0.008	3.43	0.047	8.77	0.003
Contrasts – Litter Dist.										
Uniform vs. Aggregated	1	10	0.18	0.682	6.23	0.032	1.02	0.334	11.2	0.007

† Num df and Den df denote the numerator and denominator degrees of freedom used in F tests, respectively.
‡ the denominator degrees of freedom for sampling day of year (DOY) 234 is 11, one more than the other sampling dates.

Table 5.3 Repeated measures analysis of variance to determine effects of litter distribution on litter-derived soil inorganic N (NO_3^- + NH_4^+).

Source	Num. df†	Den. df†	F value	P>F
Block	3	17	1.62	0.223
Litter distribution	3	17	1.91	0.166
Sampling Date	1	17	81.7	<0.0001
Distribution x Date	3	17	4.69	0.015

† Num df and Den df denote the numerator and denominator degrees of freedom used in F tests, respectively.

Table 5.4 Analysis of variance to determine effects of litter distribution on litter-derived soil inorganic N (NO_3^- and NH_4^+) for each sampling date during the growing season. The contrasts compare the mean effect of aggregated litter versus uniform litter distribution.

			Sampling Date (day of year)			
			------173------		------201------	
Source	Num. df†	Den. df†	F	P>F	F	P>F
Block	3	7	0.96	0.462	1.81	0.233
Litter Distribution	3	7	3.06	0.101	2.66	0.129
Contrasts – Litter Dist.						
Uniform vs. Aggregated	1	7	4.48	0.072	3.46	0.105

† Num df and Den df denote the numerator and denominator degrees of freedom used in F tests, respectively.

Table 5.5 Repeated measures analysis of variance to determine effects of litter distribution on maize shoot N content.

Source	Num. df†	Den. df†	F value	P>F
Block	3	91	2.03	0.115
Litter distribution	4	91	6.60	<0.0001
Sampling Date	6	91	272.3	<0.0001
Distribution x Date	24	91	2.08	0.007

† Num df and Den df denote the numerator and denominator degrees of freedom used in F tests, respectively.

Table 5.6 Analysis of variance to determine effects of litter distribution on maize shoot N content for each sampling date during the growing season. The contrasts compare the mean effect of aggregated litter versus uniform litter distribution.

			Sampling Date (day of year)						
			173	188	201	226	234	269	285
Source	Num. df†	Den. df†	P>F						
Block	3	10	0.183	0.918	0.472	0.071	0.025	0.380	0.169
Litter Distribution	4	10	0.880	0.900	0.993	0.309	0.008	0.001	0.166
Contrasts – Litter Dist.									
Uniform vs. Aggregated	1	10	0.551	0.969	0.688	0.409	0.130	0.285	0.109

† Num df and Den df denote the numerator and denominator degrees of freedom used in F tests, respectively.

Table 5.7 Repeated measures analysis of variance to determine effects of litter distribution on the proportion of maize shoot N derived from red clover litter.

Source	Num. df†	Den. df†	F value	P>F
Block	3	74	4.90	0.004
Litter distribution	3	74	7.52	0.0002
Sampling Date	6	74	21.1	<0.0001
Distribution x Date	18	74	1.60	0.081
Contrast				
Uniform vs. Aggregated	1	74	16.46	0.0001

† Num df and Den df denote the numerator and denominator degrees of freedom used in F tests, respectively.

Table 5.8 Repeated measures analysis of variance to determine effects of litter distribution on maize root N content.

Source	Num. df†	Den. df†	F value	P>F
Block	3	38	0.33	0.805
Litter distribution	4	38	4.76	0.003
Sampling Date	2	38	575.0	<0.0001
Distribution x Date	8	38	3.05	0.010

† Num df and Den df denote the numerator and denominator degrees of freedom used in F tests, respectively.

Table 5.9 Analysis of variance to determine effects of litter distribution on maize root N content for each sampling date during the growing season. The contrasts compare the mean effect of aggregated litter versus uniform litter distribution.

			Sampling Date (day of year)					
			-------188-------		-------226-------		------269------	
Source	Num. df†	Den. df†‡	F	P>F	F	P>F	F	P>F
Block	3	9, 11, 12	0.68	0.586	0.52	0.680	0.59	0.631
Litter Distribution	4	9, 11, 12	0.22	0.920	1.90	0.180	4.48	0.019
Contrasts – Litter Dist.								
Uniform vs. Aggregated	1	9, 11, 12	0.01	0.925	0.06	0.817	7.84	0.016

† Num df and Den df denote the numerator and denominator degrees of freedom used in F tests, respectively.
‡ the denominator degrees of freedom for each sampling date, respectively.

Table 5.10 Repeated measures analysis of variance to determine effects of litter distribution on the proportion of maize root N derived from red clover litter.

Source	Num. df†	Den. df†	F value	P>F
Block	3	30	1.90	0.370
Litter distribution	3	30	10.6	<0.0001
Sampling Date	2	30	4.92	0.014
Distribution x Date	6	30	1.43	0.238
Contrast				
Uniform vs. Aggregated	1	30	24.5	<0.0001

† Num df and Den df denote the numerator and denominator degrees of freedom used in F tests, respectively.

Table 5.11 Repeated measures analysis of variance to determine effects of litter distribution on nitrate leached from the containers.

Source	Num. df†	Den. df†	F value	P>F
Block	3	18	1.94	0.251
Litter distribution	3	18	1.30	0.305
Sampling Date	1	18	2.20	0.155
Distribution x Date	3	18	0.66	0.589
Contrast				
Uniform vs. Aggregated	1	18	0.04	0.850

† Num df and Den df denote the numerator and denominator degrees of freedom used in F tests, respectively.

Table 5.12 Repeated measures analysis of variance to determine effects of litter distribution on litter-derived NO_3^- leached from the containers.

Source	Num. df†	Den. df†	F value	P>F
Block	3	18	0.61	0.617
Litter distribution	3	18	2.62	0.083
Sampling Date	1	18	7.13	0.016
Distribution x Date	3	18	1.73	0.199
Contrast				
Uniform vs. Aggregated	1	18	0.91	0.353

† Num df and Den df denote the numerator and denominator degrees of freedom used in F tests, respectively.

Table 5.13 Repeated measures analysis of variance to determine effects of litter distribution on net litter-N mineralization.

Source	Num. df†	Den. df†	F value	P>F
Block	3	38	1.50	0.231
Litter distribution	3	38	11.7	<0.0001
Sampling Date	3	38	176.8	<0.0001
Distribution x Date	3	38	3.03	0.008
Contrast				
Uniform vs. Aggregated	1	38	31.0	<0.0001

† Num df and Den df denote the numerator and denominator degrees of freedom used in F tests, respectively.

Table 5.14 Analysis of variance to determine effects of litter distribution on net litter-N mineralized for each sampling date during the growing season. The contrasts compare the mean effect of aggregated litter versus uniform litter distribution.

			Sampling Date (day of year)							
			-------173-------		-------201-------		-------234-------		-------285-------	
Source	Num. df†	Den. df†‡	F	P>F	F	P>F	F	P>F	F	P>F
Block	3	6,6;8,9	0.47	0.716	1.90	0.230	4.43	0.041	1.22	0.357
Litter Distribution	4	6,6,8,9	3.98	0.071	0.74	0.566	7.57	0.010	12.41	0.002
Contrast										
Uniform vs. Aggregated	1	6,6,8,9	6.26	0.046	1.90	0.218	11.3	0.010	32.2	0.0003

† Num df and Den df denote the numerator and denominator degrees of freedom used in F tests, respectively.
‡ the denominator degrees of freedom for each sampling date, respectively.

Table 5.15 Percent litter-^{15}N recovery into maize shoots and roots, soil organic and inorganic N, leached N, and unexplained N loss in response to litter distribution ± one standard error at the end of the growing season.

	Litter distribution			
	8 patches	24 patches	72 patches	Uniform
Nitrogen Pool	% litter-^{15}N recovery			
Soil organic N	50.6 (4.9)	57.7 (3.4)	50.0 (1.7)	60.0 (3.3)
Soil inorganic N	0.1 (0.02)	0.2 (0.02)	0.2 (0.02)	0.2 (0.02)
Maize shoot N	27.2 (1.2)	26.0 (0.8)	25.8 (0.6)	22.5 (0.5)
Maize root N	5.7 (0.4)	4.3 (0.2)	4.5 (0.2)	2.6 (0.2)
Leached N	0.02 (0.02)	0.02 (0.01)	0.09 (0.05)	0.02 (0.01)
Unexplained N	16.4 (4.3)	11.9 (3.7)	19.4 (2.3)	14.7 (3.4)

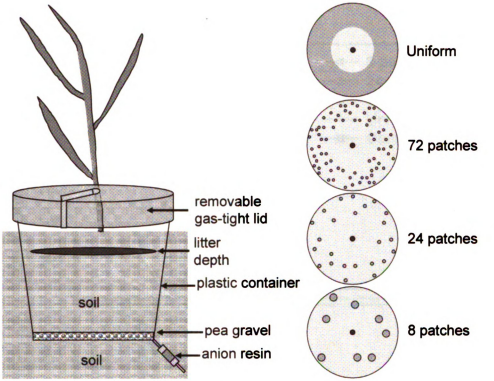

Figure 5.1 Spatial layout of litter distribution treatments conducted in 50-L plastic containers set into holes in a field and surrounded by maize plants. The left panel contains a side-cut view of the container components and vertical layout. The right panel is a top-down view illustrating the spatial distribution of the litter treatments.

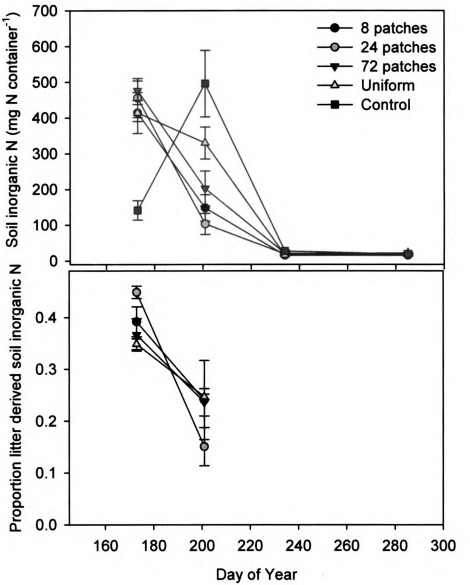

Figure 5.2 Total soil inorganic N (NO_3^- and NH_4^+) in the surface 190 mm of soil and proportion of soil inorganic N derived from soil amended red clover litter (bottom panel) in response to litter distribution. Error bars denote ± one standard error.

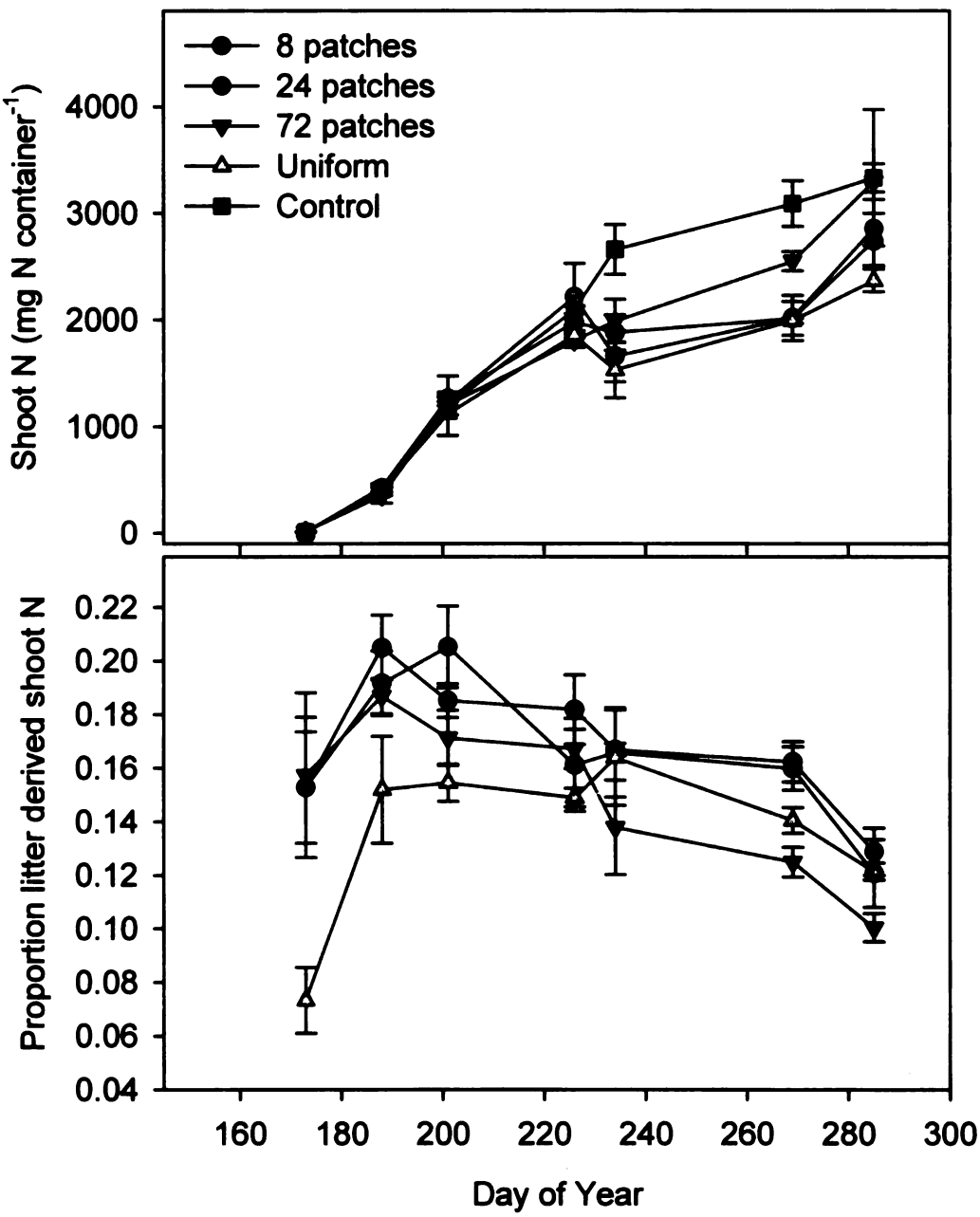

Figure 5.3 Maize shoot N content (top panel) and proportion of shoot N derived from soil amended red clover litter (bottom panel) in response to litter distribution. Error bars denote ± one standard error.

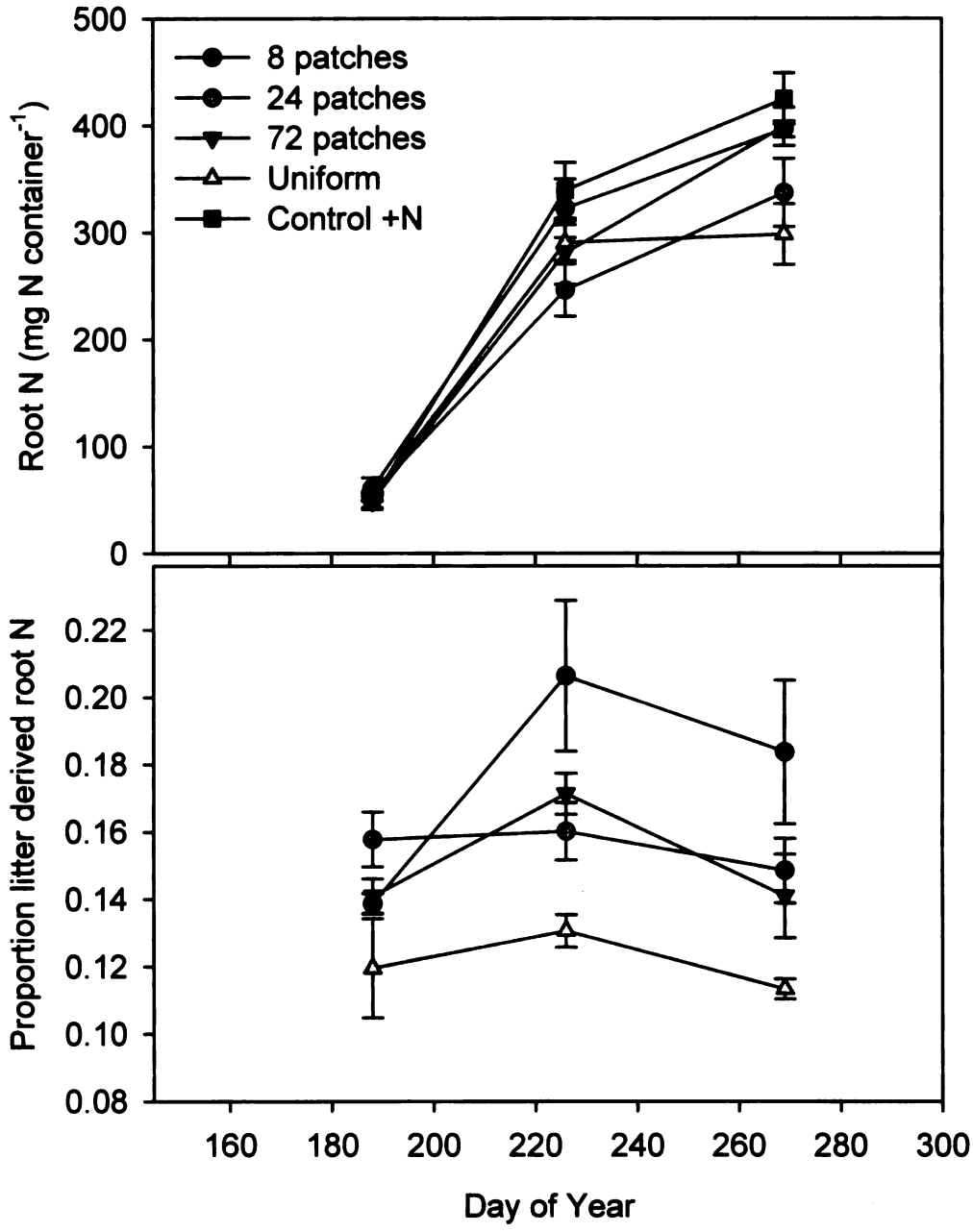

Figure 5.4 Root N content (top panel) and proportion of root N content derived from soil amended red clover litter (bottom panel) in response to litter distribution. Error bars denote ± one standard error.

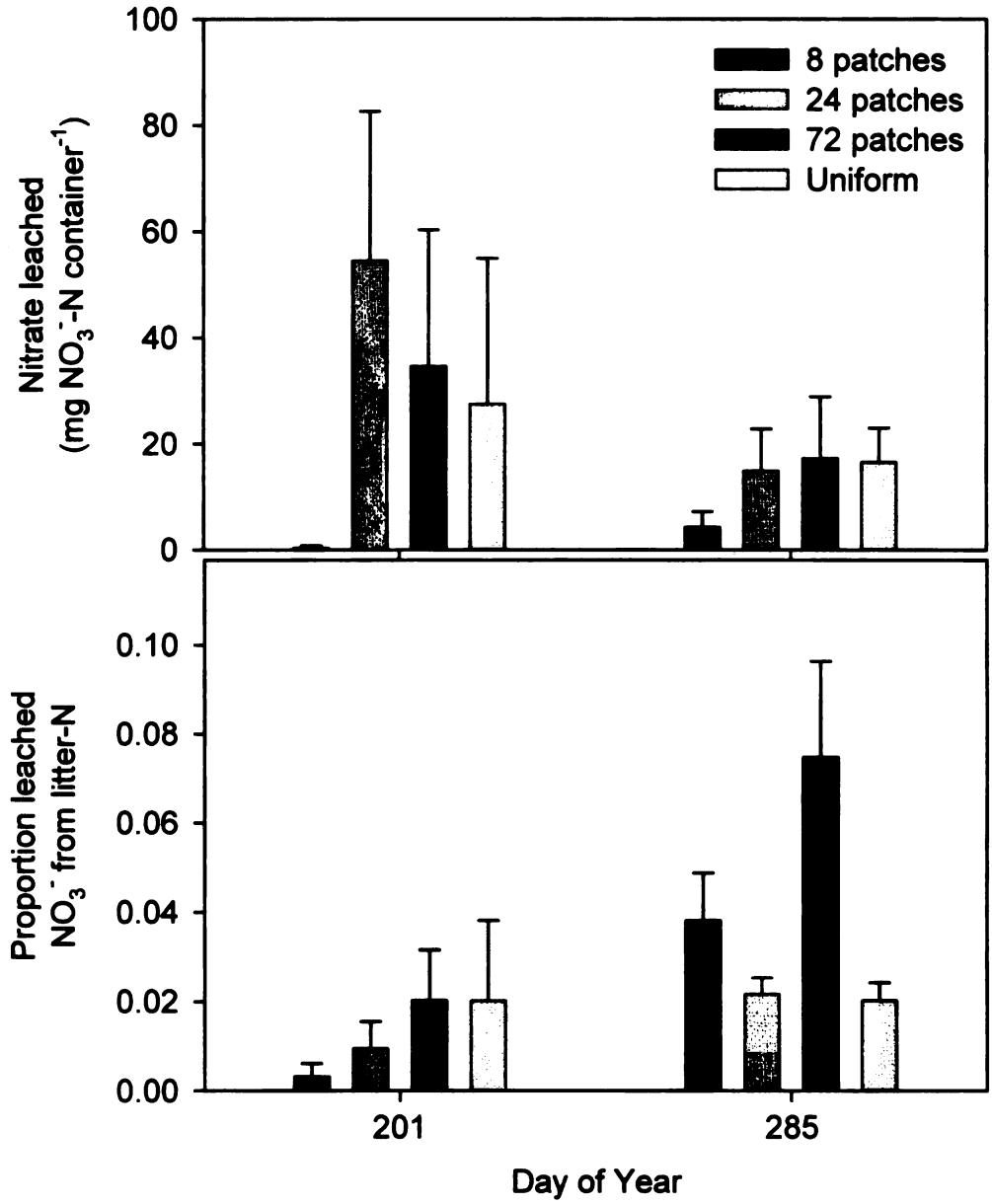

Figure 5.5 Nitrate leached and proportion of nitrate leached derived from litter-N in response to red clover litter distribution. Error bars denote one standard error.

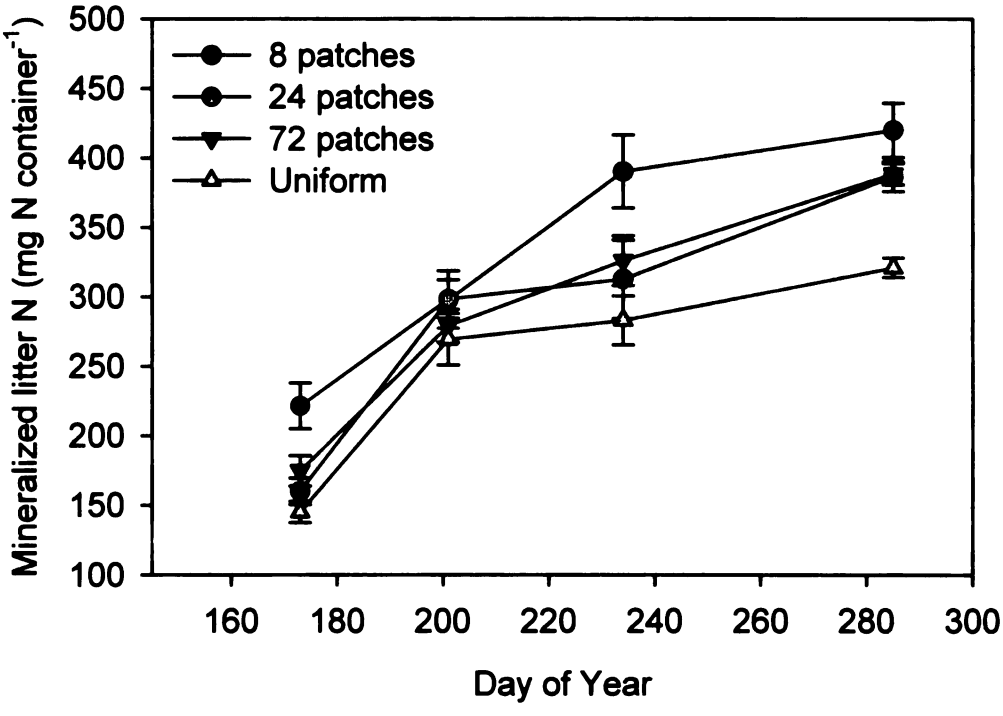

Figure 5.6 Estimated net mineralized litter-N in response to litter distribution. Error bars denote ± one standard error.

Chapter 6. Summary of Resource Aggregation Effects and Implications

SUMMARY

Spatial heterogeneity of soil resources is common whereas uniformity is rare. Jensen's Inequality predicts that process rates will differ between systems with uniform and heterogeneous distributions of process controls if the functional response to that control is nonlinear. Nonlinear microbial and plant functional responses to resource availability are prevalent in soils. Nitrogen is the most common soil derived resource limiting plant productivity in terrestrial ecosystems and plant litter is typically the most important source of soluble N compounds that are available for plant and microbial assimilation. Thus heterogeneous distributions of plant litter are likely to affect terrestrial C and N cycling differently than uniform distributions of plant litter.

Many factors that cause resource heterogeneity in soil (e.g., tillage, animal burrowing, and root distributions) also influence many other aspects of the soil environment, and thus it is difficult to attribute differences in soil process rates in natural or managed ecosystems to resource heterogeneity per se. The primary objective of my dissertation was to address the general question of how does the spatial distribution of soil microbial and plant resources within the scale of individual plants influence rates of C and N cycling. My general approach for addressing this question was to isolate the effect of resource heterogeneity by manipulating the spatial distribution of plant litter across an aggregation gradient in soil and then following specific microbial and plant responses to this

manipulation. In addition, I varied other aspects of the litter or soil environment while holding the litter aggregation constant to more fully understand the properties of litter aggregation that determine the microbial and plant mechanisms involved in these responses.

Many soil organisms have evolved adaptations to avoid, tolerate, and exploit soil resource heterogeneity. For example, plants can selectively proliferate roots or forage into microsites rich in limiting resources. Plant species vary in their root proliferation response to microsites of inorganic N; however, it is unclear if species vary in their root proliferation response to microsites of complex organic matter. Furthermore, it is unknown if plant N demand has the same control over root proliferation into microsites of organic N as into microsites of inorganic N. In Chapter 2, I addressed three questions with the overall objective to examine the soil conditions that plant root proliferation may play an important role in regulating ecosystem level C and N cycling. Specifically, I asked: 1) does root proliferation vary with microsite quality; 2) does the proliferation response correspond to increased productivity; and 3) how does the plant demand for N influence root proliferation in patches of N-rich organic matter? I addressed these questions with two experiments: one in the greenhouse and one in the field.

To address questions 1 and 2 of Chapter 2, I grew *Bromus inermis* and *Avena sativa* in sand with the only N source a choice of microsites composed of plant litter of varying N mineralization potential. The results of this experiment showed that both plant species had a similar aboveground biomass response to

the litter treatments; however, only in *A. sativa* did this response correspond to root foraging. In other words, *A. sativa* proliferated more roots into microsites that stimulated overall plant growth, whereas the root distribution of *B. inermis* was not related to the microsite resource quality.

Plant root foraging for microsites of inorganic N is thought to be controlled by plant genetics, overall soil N availability, and the plant demand for N. To address question 3 of chapter 2, I examined maize root proliferation into N-rich microsites composed of labile organic matter across an N fertility gradient. The results of this experiment showed that at about the same N fertility level that maize plants were N sufficient, as indicated by the grain yield plateau, root foraging for the microsites discontinued. Together these two experiments demonstrate that root foraging for microsites of complex organic matter can vary by plant species and that root foraging is not likely to occur for soil nutrients that the plant does not need regardless of whether the microsite holds organic or inorganic N.

The emissions of CO_2 and N_2O from litter in soils are controlled by several nonlinear functional microbial responses and thus are likely to differ between uniformly and heterogeneously distributed litter. Additionally, any alteration of C and N cycling in response to litter aggregation will potentially affect plant acquisition of litter-N. In chapter 3, I examined the influence of resource heterogeneity on microbial-derived CO_2 and N_2O emissions by manipulating plant litter across an aggregation gradient. Within this gradient we addressed four questions: 1) does the intensity of plant litter aggregation affect litter

decomposition and N_2O emissions; 2) does the aggregation effect on decomposition and N_2O fluxes vary with soil moisture and hence diffusional constraints; 3) does plant litter particle size affect CO_2 and N_2O emissions similarly when uniformly distributed and aggregated; and 4) does the presence of growing plants alter N_2O emissions in response to litter aggregation? I addressed these questions in two laboratory incubations and one field experiment.

Results showed that litter aggregation temporarily delays litter decomposition by 5 to 7 days regardless of whether the litter is finely ground or cropped into ~ 5 mm long pieces. By incubating the litter under near-water-saturated conditions (80% WFPS), I was able to infer that O_2 supply to the interior of the litter aggregates was likely limiting the initial decomposition of the aggregated litter relative to the uniform litter distribution. The most significant finding of these experiments showed that litter aggregation stimulated N_2O emissions by an average of 7 fold relative to the uniform distribution.

The microbial responses to litter aggregation suggests that N availability for plant uptake and productivity may be negatively affected by litter aggregation. In Chapter 4, I employed two litter aggregation gradients to address these questions: 1) does the aggregation of plant litter influence the growth of an individual maize plant in an N-limited system; 2) is the root to shoot ratio and belowground C allocation altered by resource aggregation, and 3) does the distribution of aggregated resources influence maize productivity? I addressed these questions in two field experiments.

First, maize productivity was stimulated by the aggregation of red clover litter in both experiments. Whereas cereal rye litter aggregation did not affect maize productivity. The litter distribution did not influence root to shoot biomass allocation of maize; however, the root-induced soil respiration was decreased as a result of litter aggregation. The changes in belowground C allocation appeared to precede changes in the aboveground and belowground biomass. I suggest that this extra expenditure of plant C may have caused the plants growing in soil with the uniform litter distribution to be smaller at the end of the season.

Second, to understand how litter aggregation alters plant N acquisition and N retention in soils I distributed ^{15}N-labelled red clover litter across an aggregation gradient in soil and followed the fate of the litter-N into the plants, soil inorganic N pools, soil organic N pools, and leachate N. With this approach I addressed three specific questions: 1) does resource aggregation influence above and belowground plant N acquisition; 2) does resource aggregation influence litter-derived net N mineralization; and 3) does resource aggregation influence whole system N retention.

Results showed that litter aggregation substantially altered N cycling in this system. The litter aggregation gradient had statistically significant but minor effects on total plant N content; however, plants growing in soil with aggregated litter derived 20% more N from the litter than plants grown in soils with a uniformly distributed litter. This difference in litter-derived plant-N is in contrast to the greater plant available inorganic N pools observed in response to the uniform distribution on two dates. Taken together these two observations suggest that a

closer spatial coupling of roots and aggregated litter allowed for greater litter-N uptake while minimizing the risk of NO_3^- leaching.

IMPLICATIONS

The overall results of these experiments suggest that the aggregation of plant litter has important effects on soil microbial processes and plant productivity that lead to significant alterations of C and N cycling. Although, litter aggregation had a positive effect on plant productivity it also stimulated N_2O emissions, and thus presents a tradeoff in services provide by agricultural ecosystems. These general implications logically follow: first, these results should inform our experimental approaches; second, results have important implications for how we manage ecosystem services; and third, these results can help us predict how changes in resource distribution and availability at spatial scales of less than 1 m will alter ecosystem level process rates in managed and natural ecosystems.

We commonly study plant-soil interactions and their influence in ecosystem level processes in one of two manners: 1) under homogenized soil conditions so we can confidently conclude that the factor we manipulated has a consistent effect on the plant or soil processes across each our experimental replicates, or 2) under in-situ conditions where many different factors are varying at the same time. This balance between deterministic manipulation and realism is difficult and is also part of what makes ecology experimentally challenging. My deterministic isolation of a single component of soil heterogeneity, i.e. litter aggregation, is a small step towards explaining the influence of heterogeneity in the environment. My results; however, suggest that even at the sub plant scale

homogenized environmental conditions may lead to erroneous conditions regarding ecosystem level C and N cycling. The physical description of my system should be translatable into a mathematical model to be used to further explore the influence of resource aggregation on C and N cycling.

The temporal scale of my experiments was on the order of days to growing seasons. This time scale may have important implications for my interpretations. For example, from 40 day incubations I concluded litter aggregation had only minor effects on decomposition; however, on the time scale of years to decades the litter distribution may alter C storage in manners not predicted from the first weeks of decomposition. For instance, the distribution of litter in soil may have differential effects on mycorrhizal associations. I would predict that a uniform litter distribution would promote mycorrhizal associations because the perceptual scale at which fungal hyphae detect nutrient rich microsites is likely smaller than that of plant roots. Increased mycorrhizal dependence has been associated with greater C sequestration rates, so potentially a longer term study would demonstrate that soil organic C may increase under management to promote uniformity.

Management of agricultural inputs has become more spatially sophisticated during the last two decades with the coupling of global positioning systems and spatial data on soil properties. Most of this work has focused on spatial scales of 5 m and larger. Management practices that manipulate sub plant scale resource distribution are as old as agriculture itself. Modern day management of crop, cover crop, and weed litters starts with the chopper box

inside the combine, followed by flail choppers and mowers, then tillage and planting followed by interrow cultivation. Each of these operations alters the litters' particle size and horizontal and vertical distribution. Because so many of these management strategies are conducted to achieve multiple objectives, it is difficult to relate any one practice to the differences yield or N_2O emission that my results suggests might be related to litter distribution. However, a first step towards understanding this possibility would start by increasing or decreasing the intensity of one of these practices.

In unmanaged ecosystems, my results suggest that heterogeneity of resource distribution may manifest itself in many ways. For example, herbivorous insects within the same community can vary in body size by several orders of magnitude, for example aphids and gypsy moths in oak-hickory forests. Lets assume that we have a 1000 aphids m^{-2} and 5 gypsy moths m^{-2} and the biomass of each is the same. As these insects feed, their frass falls to the soil surface as clumps in proportion to their mass body. This frass then becomes a resource for soil organisms and potentially for the plants that the insects originally feed upon. From my dissertation research, I would predict that the aphid frass although of equal resource quantity to the soil microbes would be less likely to stimulate N_2O emissions than the decomposing gypsy moth frass because smaller clumps of frass are less likely induce anaerobic conditions. Furthermore, if the plants in this community varied in their root foraging for patches of different resource quantity then the plant species may differentially benefit from the presence of one herbivore over the other through an indirect

interaction of their decaying frass. If we had assumed that mean resource quantity per unit of area controlled the N_2O emissions rate instead of the distribution of that resource then we would have underestimated the N_2O emissions from forests with gypsy moths and aphids versus that forest without gypsy moths.