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REGULAR ARTICLE

Soil resource heterogeneity in the form of aggregated litter alters maize productivity

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Abstract Soils are spatially heterogeneous. To better understand the effects of sub-plant scale soil resource heterogeneity on primary productivity we aggregated plant litter (*Trifolium pratense* L. shoots and *Secale cereale* L. leaves) into 8, 24, 32, and 72 patches and uniformly distributed in 50-L containers (0.15 m^2) of soil and grew *Zea mays* L. throughout two seasons. On average aggregated *T. pratense* litter enhanced final aboveground maize biomass by 14% relative to uniformly distributed *T. pratense* litter. This effect may be related to the reduction in root carbon allocation observed as lower apparent root respiration rates compared to uniform litter distribution. In contrast, the spatial distribution of *S. cereale* litter did not affect productivity. The common experimental approach of uniformly distributing resources to understand their influence on soil-plant processes likely oversimplifies field conditions because our results indicate that the spatial distribution of plant litter alone can influence productivity and plant carbon allocation.

Keywords Spatial heterogeneity · Root foraging · Nitrogen mineralization · Root respiration · Soil surface CO_2 flux · Corn

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Introduction

Heterogeneity is a universal characteristic of natural and managed soils, but its functional influence at the sub-plant scale on primary productivity remains poorly understood. Variation in plant growth responses to soil resource heterogeneity is likely attributable to soil resource characteristics, environmental 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 having to forage for P (Kume et al. 2006; Zhu et al. 2005). In contrast, the spatial aggregation of nitrate (NO_3^-), a

more mobile plant nutrient in most soils, also stimulates root proliferation (Drew 1975), but with inconsistent effects on productivity (Robinson 1994). One reason for this may be that NO_3^- acts as a signal to initiate root proliferation (Zhang et al. 2007) in unfertilized systems due to the association of NO_3^- 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 then 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 micro-sites 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 conversely, less root biomass is required to meet the nutritional demands of the plants. On the whole plant scale, optimization principles suggest that the root to shoot (R:S) biomass ratio should decrease in plant-soil systems that are acquiring soil nutrients more efficiently (Hutchings and John 2004).

In agricultural ecosystems, a shift in the R:S ratio due to changes in nutrient uptake efficiency will be manifested in altered grain yield. Tests of agricultural crop responses to sub-plant scale spatial soil resource heterogeneity are abundant for inorganic forms (e.g., NO_3^- and NH_4^+), but the influence on crop productivity when organic matter is the heterogeneously distributed source of plant N availability is largely unknown. Here we test the hypothesis that the extent of resource aggregation and quality (e.g., C:N ratio) alters maize productivity, R:S ratio, and belowground C allocation in a system limited by N availability. We address this hypothesis with two experiments. The first experiment addresses the question of how does resource aggregation and quality affect maize productivity, and the second experiment both replicates the first experiment with a single resource type and explores in greater detail whole plant responses to heterogeneous resource distribution.

Materials and methods

We conducted these studies at the W.K. Kellogg Biological Station (KBS) in Southwest Michigan, USA (42° 24'N, 85°24'W, elevation 288 m). In experiment 1, we examined the effect of clustered plant litter aggregation on maize productivity. In contrast, in experiment 2 we studied the influence of randomly distributed litter aggregates on aboveground and belowground plant responses. 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. 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. The Ap horizon of the Kalamazoo series is~30 cm deep and contains 43% sand, 38% silt, and 19% clay with a cation exchange capacity (CEC) of~8.4 cmol (+) kg^{-1}, 12.85 g C kg^{-1}, 1.31 gN kg^{-1}, and a pH of 5.5. The Ap horizon of the Oshtemo series is~30 cm deep with 59% sand, 27% silt, and 14% clay and contains a CEC of~7.1 cmol (+) kg^{-1}, 9.67 g C kg^{-1} and 1.04 gN kg^{-1} with a pH of 5.7 (Crum and Collins 1995). We excavated soil from the field, mixed it with an end-loader, placed it in a tarp covered pile to air dry, and after 12 months we power sieved the soil through a 12 mm screen, and combined it with sand in a 250-L mixer. This soil mix was used for both experiments.

Clustered litter distribution–experiment 1

The clustered litter distribution experiment was laid out in a randomized complete block design (RCBD) with six soil amendment treatments, five sampling dates, and four replicates (6×5×4=120 experimental units). We included an additional no N added control treatment sampled only on the final harvest date (n=4 replicate units) for a total of 124 experimental units. An experimental unit comprised of a 50-L black plastic container filled with the soil mix, amendment, and a single maize plant (Pioneer ® 35Y54) (Fig. 1). The six soil amendment treatments consisted of red clover (*Trifolium pratense* L.) distributed into three spatial distributions, rye (*Secale cereale* L.) distributed into two spatial distributions, and a $NaNO_3$ fertilizer control (control+N) and no N added control (control -N) treatments.

Litter consisted of red clover and 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, and ground to pass a 1-mm screen in a Cyclotec® 1093 sample mill (Tecator, Sweden). Eight subsamples of each finely-ground litter were analyzed for C and N content with a Costech ECS 4010 CHN elemental analyzer (Valencia, CA, USA). The red clover litter contained 410 g C kg litter^{-1} and 29.8 gN kg litter^{-1}, for a C:N ratio of 13.7, and the rye contained 392 g C kg litter^{-1} and 16.1 gN 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 eight patches or distributed uniformly (Fig. 1).

Litter application

Litter distribution treatments were constructed by placing a 15-cm diameter x 15 cm length polyvinyl-chloride (PVC) cylinder flat onto Teflon® coated metal sheets. Into the PVC cylinder we added 600 mL of soil mix, then a template for the aggregated litter treatments. The template was a 14.5 cm diameter circular sheet of acrylic with four 21.3-mm or sixteen 8.3-mm diameter plastic cylinders adhered perpendicular to the sheet. We used syringe plungers to displace a consistent volume of soil within the cylinders before adding litter to the cylinders. We added 300 mL of soil around the template and then pushed the plungers

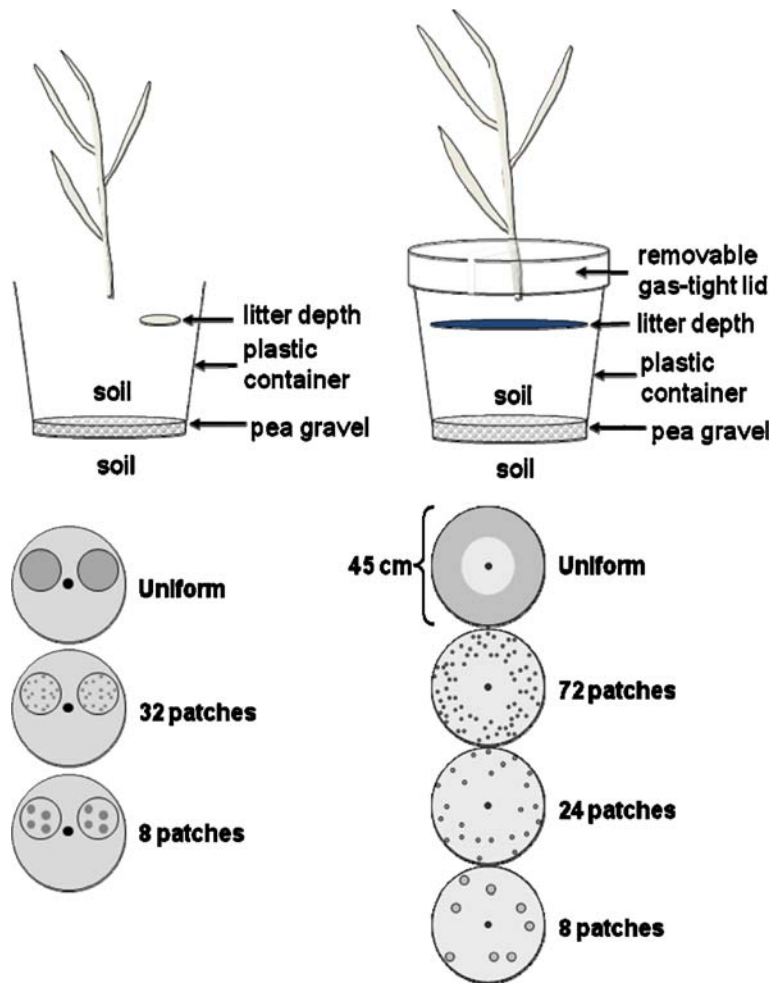

Fig. 1 Schematic depiction of the spatial layout of litter distribution treatment for the clustered (Experiment 1, left side) and random (Experiment 2, right side) distribution experiments conducted in 50-L plastic containers set into pits in the soil and surrounded by other maize plants. In the top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

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 approximately density (~0.4 g of litter mL^{-1}) and mean depth in the soil (Fig. 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 28.87−g) and 36.02 g (49.00−g or uniformly 36.02−g), respectively.

Field containers, planting and fertilization

We prepared 50-L (37 cm in depth, 39 cm bottom diameter and 44 cm top diameter) containers for this experiment with all drainage directed through a single outlet. We added 3 L of ~1 cm diameter gravel to the bottom of each container to aid drainage, lining of medium weight landscaping fabric to avoid soil loss, and 40 L of soil mix. Containers were placed into the field so that soil surface inside and outside of the containers were at the same elevation and at least 2.5 m from other containers. We chose this container size because it has a surface area of about 0.15 m^2, which is similar to 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.

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 (Fig. 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 2 cm from the top of the container and then the PVC cylinders were removed.

We designed this experiment to test maize 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 (2 g P L^{-1}, 5 gK L^{-1}, 1 gS-SO_4^{-2} L^{-1}, 1 g Ca L^{-1}, and 0.3 gMg L^{-1}) to the surface of each container. On June 29, 2005, we transplanted 2 d old maize seedlings into the center of each container and placed 6 mm screen hardware cloth over the containers until the seedlings were at the V2 growth stage (Hanway 1963) to protect them from rodents. The control+N treatment was implemented on July 8, 2005, by adding 1 L solution of 1.6 gN-$NaNO_3$ L^{-1} 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.

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. The soil, roots, and litter of the surface 19 cm and the soil and roots of the bottom 18 cm of each container was separately sieved to pass a 6 mm screen, weighed wet, and ~350 g sub-samples were dried (4 d at 65°C), and weighed.

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 (Fig. 1). Additionally, we quantified belowground biomass and root induced soil CO_2 flux as well as the aboveground biomass. 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 no landscape fabric was used. We distributed the red clover litter (shoots) into 8, 24, or 72 patches or uniformly in the soil except no litter was placed within the center 15 cm of the container to avoid disturbing the litter patches when planting the maize. The red clover litter contained 413 g C kg $litter^{-1}$ and 31.0 gN kg $litter^{-1}$ for a C:N ratio of 13.3.

Litter application

The litter application method was modified from experiment 1. We applied the litter directly to soil in the containers with a template that was the same diameter as the inside of the container (Fig. 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 10 cm below the soil surface. The litter application process lasted 3 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) at a depth of ~5 cm into the center of the each container on May 31 and covered the containers 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 in experiment 1, on July 6, 2006 and 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 both aboveground and belowground plant growth from 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 later separated the maize roots from other detritus, soil particles, and any remaining clover litter and 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 with and without growing maize plants (Hanson et al. 2000). To accomplish this, additional 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 was sealed around the outside of the 50-L container and the plant stem (Fig. 1). The chamber lids were modified 120-L refuse containers and lids with a 6 cm wide slit removed from one 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 min and the chamber lids remained on the container for a maximum of 70 min during the 4 gas samples were taken. 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. Gas samples were analyzed for CO_2 concentration using an infrared gas absorption analyzer (LI-Cor 820). 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

Both experiments were analyzed with a repeated measures analysis of variance (ANOVA) with block and soil amendment as independent fixed effects and the sampling date as 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 variance assumption of ANOVA. When the interaction of main effects and sampling date were significant ($P<0.05$), 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. Effects were considered significant at the $P<0.05$ level for all ANOVAs.

Results

Experiment 1: plant growth response to clustered litter distribution

By the end of the growing season each of the clustered litter amendments stimulated maize aboveground growth as compared to the control–N treatment, but the litter treatments were not as productive as the control+N treatment (Fig. 2 and Table 1). This indicates that maize plants in litter-amended containers were N-limited; however, this N limitation was not apparent until the end of the growing season. At the final harvest, maize aboveground biomass was 13% greater when red clover litter was aggregated (119±4.9 g plant^{-1}, average of 8 and 32 patch treatments) versus uniformly distributed (105±4.9 g plant^{-1}). In contrast, rye litter spatial distribution did not affect aboveground maize biomass. The degree of red clover litter aggregation (8 vs. 32 patches) affected aboveground productivity similarly in this experiment.

Soil moisture content in the surface 19 cm and in the bottom 18 cm 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

Aggregated red clover (8, 24, and 72-patch treatments) consistently stimulated maize aboveground biomass production more than uniformly distributed red clover litter (Table 2). However, the differences among litter distribution treatments were most noticeable during the grain filling growth stage (Fig. 3). At physiological maturity, control+N treated containers produced similar aboveground maize biomass responses as the aggregated litter treatments, whereas plants grown in containers treated with a uniform litter distribution were 25% smaller than the control+N. The aggregation of litter promoted final aboveground biomass by 16% over the uniform treatment (315 vs. 272±16 g plant^{-1}). Additionally, there were no detectable differences in final aboveground biomass among the aggregated litter treated soils.

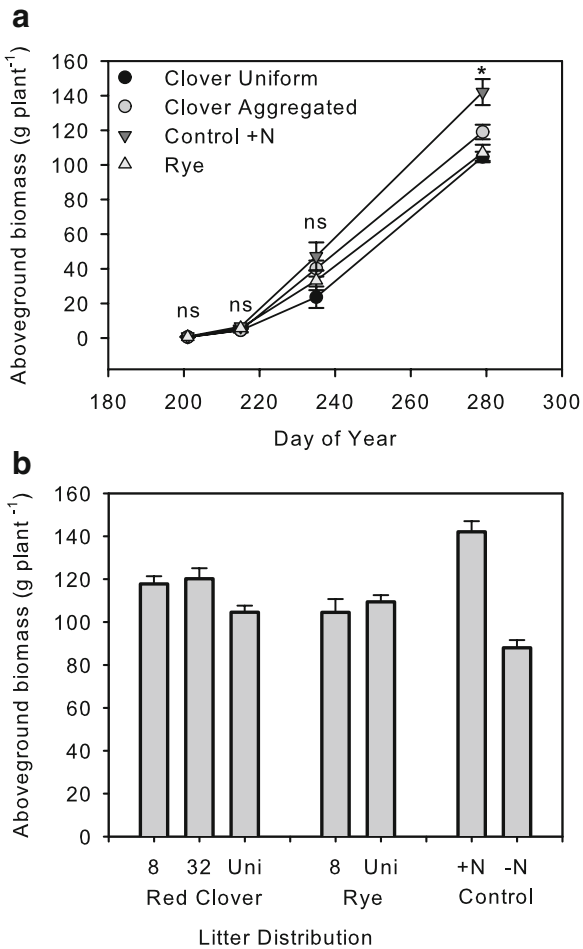

Fig. 2 Aboveground biomass response to the distribution of rye and red clover litter and+N and–N control soil amendments during Experiment 1. Panel A depicts the change in aboveground biomass throughout the season for the average of the rye litter, average of the aggregated red clover litter, uniformly distributed red clover litter, and the control plus N fertilizer treatments. Symbols above data from each sampling date represents statistical significance, ns=$P>0.05$ and *=$P<0.05$. Panel B depicts the final aboveground biomass of each of the soil amendment treatments for experiment 1

Table 1 Analysis of variance of soil amendments effects on aboveground maize biomass for each sampling date during Experiment 1

			Sampling Date (day of year)			
			201	215	235‡	279
Source	Num. df†	Den. df†	*P*>*F*			
Block	3	15	ns	ns	ns	ns
Soil Amendment	5	15	ns	ns	ns	***
Contrasts						
Clover litter Dist.	1	15	ns	ns	ns	*
Clover–8 vs. 32	1	15	ns	ns	ns	ns
Rye litter Dist.	1	15	ns	ns	ns	ns
Litter species×Dist.	1	15	ns	ns	ns	*

† 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

ns, *, and *** indicate significant differences at the $P>0.05$, $P<0.05$, and $P<0.001$ levels, respectively

Root biomass response to litter distribution was similar to aboveground biomass. Root biomass increased with time and litter distribution effects were only detectable at the final root biomass sampling (Fig. 3). After physiological maturity, the mean root biomass of the aggregated litter treatments was 26% greater than the uniformly distributed litter. With similar responses above and belowground, the R:S ratio was similar among all soil treatments 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 19 cm 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; however, neither soil depth was affected by soil treatment (data not shown, $P>0.05$).

Table 2 Repeated measures analysis of variance of soil amendment effects on aboveground maize biomass during Experiment 2

Source	Num. df†	Den. df†	P>F
Block	3	81	ns
Soil amendments	4	81	**
Sampling date	5	81	***
Soil Amend.×Date	20	81	ns
Contrast			
Uniform vs. Aggregated	1	81	*
Uniform vs.+N control	1	81	***
Aggregated vs.+N control	1	81	ns

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

ns, *, **, and *** indicates significant differences at the $P>0.05$, $P<0.05$, $P<0.01$, and $P<0.001$ levels, respectively

Root induced soil surface CO_2 flux

In the absence of plants, adding uniformly distributed red clover litter to soil increased the surface soil CO_2 flux by 22% on average throughout the sampling period over the control soil (20.7±1.2 vs. 17.0± 1.2 mg C $m^{-2}h^{-1}$; Fig. 4). With plants in the system CO_2 fluxes were higher at each sampling date than without plants and this plant influence was more apparent as the season progressed. The planted aggregated litter treatments (8, 24, and 72 patches) affected soil surface CO_2 flux similarly throughout the sampling period with an average of 57.3±4.4 mg C $m^{-2}h^{-1}$. On average this was 29% lower than the CO_2 flux rate from the planted uniformly distributed litter (80.7±4.3 mg C $m^{-2}h^{-1}$, Table 3). The CO_2 flux differences among the planted litter distributions arose mainly from three sampling dates: DOY 170, 208, and 217. During the latter two dates, this effect was due to the apparent differences in root induced respiration that were 35% and 48% lower in response to aggregated litter than uniformly distributed litter. The apparent root induced respiration from the uniform litter treatment accounted for 74% of the total CO_2 flux across the entire sampling period and 82% of the flux during the last four sampling dates.

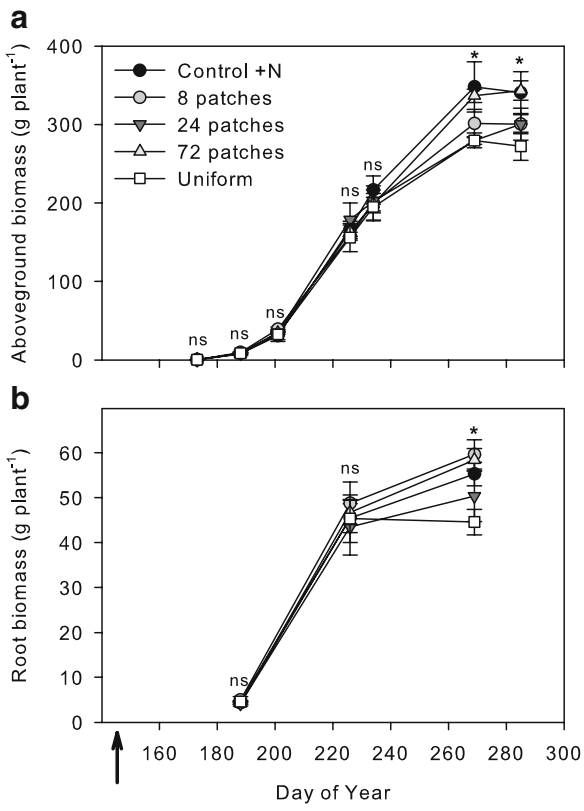

Fig. 3 Aboveground (**a**) and belowground (**b**) biomass response to red clover litter distributed into 8, 24, or 72 patches or uniformly and+N control soil amendments during Experiment 2. Symbols above data from each sampling date represents statistical significance for the contrast between aggregated and uniform litter distributions, 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

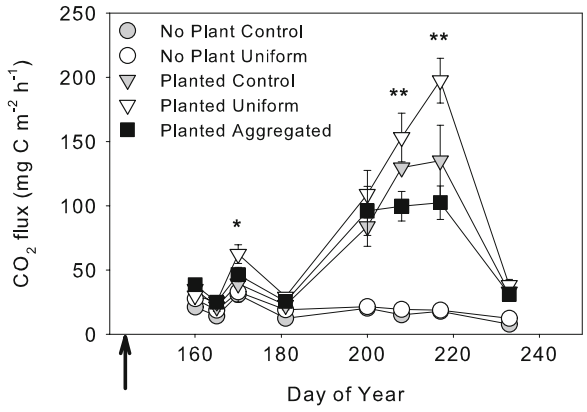

Fig. 4 Soil surface CO_2 flux with and without maize plants and with no litter, uniform litter, or aggregated litter (8, 24, and 72 patches) during Experiment 2. Error bars denote one standard error of the mean CO_2 flux. The repeated measures ANOVA of the containers with growing plants indicated no significant litter distribution x sampling date interaction and significantly greater CO_2 flux from uniform vs. the average of aggregated litter treatments. * and ** indicate significant contrast differences between planted aggregated and planted uniform litter distribution treatments at the $P<0.05$ and $P<0.01$ levels. The arrow on the x-axis denotes the time of container deployment to the field

For the plant aggregated litter treatments the same statistics were 51% and 75%. On average (all planted treatments), the apparent root induced respiration accounted for 76% of the total CO_2 flux during the last four sampling dates and 66% across the entire sampling period.

Discussion

Our results demonstrate that sub-plant scale spatial heterogeneity of labile organic N can influence above and belowground productivity of field grown maize. However, this plant growth response varied with resource substrate (i.e., litter species). Although our two experiments resulted in drastically different aboveground productivity due to differences in growing season length (Figs. 2 and 3), on average across these experiments we observed a 14% increase in aboveground maize production when red clover litter was distributed into discrete patches compared to a uniform distribution. This effect size is substantially lower than Bonkowski et al.'s (2000) 55% increase in aboveground ryegrass (*Lolium perenne* L.)

Table 3 Repeated measures analysis of variance of soil amendment effects (randomly distributed litter aggregates and+N control) on soil surface CO_2 flux from containers with maize plants during Experiment 2

Source	Num. df†	Den. df†	$P>F$
Block	3	110	ns
Soil amendment	4	110	*
Sampling date	7	110	***
Soil amend.×date	28	110	ns
Contrast–litter distribution			
Uniform vs. aggregated	1	110	**
Uniform vs. control+N	1	110	ns
Aggregated vs. control+N	1	110	ns

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

ns, *, **, and *** indicates significant differences at the $P>0.05$, $P<0.05$, $P<0.01$, and $P<0.001$ levels, respectively

biomass response to layers of chopped ryegrass litter, but greater than many experiments where inorganic N patches were distributed heterogeneously versus uniformly (as reviewed by Robinson 1996).

Several non-mutually exclusive mechanisms related to litter characteristics and plant root physiology and anatomy may account for the observed influence of litter distribution on plant growth (Fitter et al. 2000). First, the litter distribution may interact with physiochemical and biological processes in the soil to alter the timing or magnitude of N mineralization from the litter. For instance, relative to a uniform distribution, a heterogeneous distribution of red clover litter has been found to increase N mineralization due to a reduction in soil mineral-litter interactions (Breland 1994) and delay peak litter decomposition rate by 5 to 7 days because of anoxic conditions in litter patches (Loecke and Robertson 2009). We were unable to detect a similar litter aggregation effect by monitoring the soil surface CO_2 flux, likely because we did not begin field measurements of CO_2 until~16 days after litter application.

Second, litter characteristics including C:N ratio may interact with spatial distribution to differentially affect decomposition and N mineralization. For example, the rye litter we used in experiment 1 had a higher C:N ratio than the red clover litter (24.4 vs. 13.7), and thus based on stoichiometry alone the red clover is likely to at least initially decompose and mineralize N at a faster rate than the rye litter. Biological O_2 demand is proportional to the decomposition rate and thus the rapidly decomposing patches of red clover litter are more likely to become anaerobic as they exhaust soil O_2 supplies faster than patches of rye litter (Loecke and Robertson 2009). If this is so, aggregating red clover may affect soil N cycling more than aggregating rye litter. Support for this idea comes from Bonkowski et al. (2000), who found the spatial distribution of ryegrass litter (C:N of 22) did not affect its decomposition rate, whereas Loecke and Robertson (2009) that found that aggregation of red clover influenced its decomposition and nitrous oxide emission rates.

Third, aggregating litter may affect plant root foraging and nutrient uptake efficiency. Maize biomass R:S ratios were unaffected by red clover litter distribution in experiment 2 (Fig. 3). This indicates that the differences in the apparent N limitation among aggregated and uniformly distributed litter were insufficient to cause a shift in whole-plant biomass allocation. The lack of maize R:S ratio response was somewhat surprising because maize is often cited as having a plastic R:S ratio depending on above and belowground resource availability (Amos and Walters 2006); however, with coefficients of variation ranging from~10 to 20 %, our statistical power for detecting difference in R:S ratio was not strong.

Fourth, root respiration, an additional belowground C allocation, may also be altered by soil nutrient supply and the chemical form of nutrients (e.g., NO_3^- vs. NH_4^+) (Granato et al. 1989). Around the time of maximum maize root biomass we observed greater soil surface CO_2 flux rates from soils with a uniform litter distribution than aggregated litter (Fig. 4). Soil surface CO_2 flux has several potential sources, primarily including microbial oxidation of soil organic matter and root-derived C, carbonates, and plant root and mycorrhizal fungi metabolic activity (see review by Hughes et al. 2008), so the increase in CO_2 flux observed in the planted containers is some combination of these sources. The difference between the planted and unplanted containers at this point in the season is likely an indication of greater root C allocation to metabolic and/or exudation processes. At the plot level, N limitation tends to increase total soil CO_2 fluxes in maize systems (Ding et al. 2007). One potential source of this CO_2 is the up regulation of metabolic processes related to N uptake kinetics in response to inorganic N concentrations. In general, low soil NO_3^- concentrations are thought to induce a series of high affinity transport systems specific for NO_3^- uptake in roots, whereas high soil NO_3^- concentrations suppress this metabolically costly uptake mechanisms and promotes a constitutive low affinity transport system (Tischner 2000). A compensatory feedback system dependent on plant N demand and supply regulates the overall distribution of these uptake mechanisms throughout the root system.

Given that the uniform litter distribution likely caused a more even soil inorganic N concentration relative to the aggregated treatments, could the up regulation of the high affinity transport systems be operating over a greater proportion of the root system in the uniform litter system, especially as N limitation processes? And could this be related to the differences in CO_2 soil surface flux among the litter distribution treatments? If so, this would be consistent with the

hypothesis that nutrient uptake efficiency increases as soil nutrients are concentrated into limited volumes of soil (Weligama et al. 2008). Plant root foraging may spatially couple microsites of nutrient mineralization (N-rich microsites) and plant nutrient uptake, further increasing nutrient uptake efficiency (Bonkowski et al. 2000; Wang and Bakken 1997).

Alternatively, the increased CO_2 flux from uniform vs. aggregated litter may be related to differences in root induced microbial oxidation of the native soil organic matter or the added litter (the so called priming effect); however, it is unclear if N availability would differentially affect soil priming (Cheng et al. 2003; Kuzyakov 2002). Or the spatial distribution of resources may alter the flow of reduced plant C to root associated mycorrhizal fungi (Aikio and Ruotsalainen 2002), which may also affect litter decomposition rates (Hodge 2001). Further work is clearly needed for understanding the role of sub-plant scale resource heterogeneity on soil surface CO_2 flux because studying plant-soil systems with an artificially homogeneous soil may lead to errors in interpreting CO_2 flux and N cycling in many ecosystems.

The heterogeneity of agricultural soils is well characterized at scales ranging from 1 m to 1 km (Robertson and Gross 1994); 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, NH_4^+, and NO_3^- (Granato and Raper 1989; Zhu et al. 2005) and, for example, are reportedly 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. Our spatial manipulation of litter in these experiments is undoubtedly a simplification of reality compared to natural or agricultural ecosystems; however, it is likely that management operations (e.g., tillage) impart a similar range in litter distribution as in our experiments.

Conclusions

Aggregated red clover litter enhanced aboveground maize production by 14% relative to uniformly distributed litter regardless of whether the litter was clustered (occupying 23% of the soil surface) or randomly distributed (occupying 88% of the soil surface); however, the distribution of rye litter did not affect maize productivity. Furthermore, the intensity of red clover litter aggregation (i.e., the number or size of the litter aggregates) had no affect on maize aboveground or belowground biomass or apparent C allocation. These results suggest that under N limited conditions the common experimental approach of uniformly distributing soil amendments to understand their effect on soil-plant processes may not be as representative of field conditions as previously thought. Red clover litter distribution did not affect maize root:shoot ratios, but did influence the soil surface CO_2 flux just prior to the detection of aboveground N limitation, with the uniform treatments emitting more CO_2 across the soil surface than aggregated red clover litter. This difference in apparent root induced soil respiration indicates that soil nutrients (likely N) were acquired more efficiently from aggregated litter than uniformly distributed litter (i.e., the belowground C allocation to aboveground biomass ratio was greater for uniform than aggregated treatments). The applied implications of these findings are likely most important for systems that rely heavily on N mineralization from soil organic matter, crop residues, and organic soil amendments (e.g., certified organic production systems). In conclusion, our results suggest that the intentional manipulation of sub-plant scale heterogeneity of labile organic N should be investigated as a means to improve crop productivity.

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