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Soil resource heterogeneity in terms of litter aggregation promotes nitrous oxide fluxes and slows decomposition

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ABSTRACT

To better understand the role of resource heterogeneity in decomposition and nitrous oxide (N_2O) flux we systematically altered the degree of plant litter aggregation in soil, from uniformly distributed to highly aggregated. In laboratory incubations, we distributed 4.5 g of dried clover shoots (*Trifolium pratense* L.) in two particle sizes (1 or >5 mm) into 1, 3, or 9 patches versus uniformly distributed. Soil moisture content was also varied to manipulate soil oxygen (O_2) concentrations. In moist soil (50% water-filled pore space, WFPS), litter aggregation delayed the peak litter decomposition rate by 3–5 days compared to uniformly distributed litter regardless of the litter particle size. In contrast, under near-saturated soil conditions (80% WFPS) litter aggregation suppressed decomposition throughout the 26-day incubation period. This significant interaction between litter aggregation and soil moisture treatments suggests that diffusion of soil resources (likely O_2) plays an important role in the influence of litter aggregation on decomposition. Specifically, O_2 diffusion may more adequately meet O_2 consumption rates when litter is distributed than when aggregated. In contrast to the temporary influence of aggregation on litter decomposition, N_2O fluxes under 50% WFPS conditions were consistently greater and on average 7.9, 7.2, and 4.7-fold greater than fine aggregated litter (1, 3, and 9 patches, respectively) than when uniformly distributed. Coarse litter aggregation also stimulated N_2O emissions, but not as much as fine litter. Under field conditions with growing maize (*Zea mays* L.), litter aggregation also stimulated N_2O emissions. The results suggest that litter aggregation plays a role in N_2O flux from agricultural soils and it might be manipulated to provide an additional N_2O mitigation strategy.

1. Introduction

As a potent greenhouse gas, nitrous oxide (N_2O) is responsible for about 6% of the current greenhouse effect, and it is an agent of stratospheric ozone degradation (IPCC, 2007). Globally, agricultural soils account for about 60% of the atmospheric N_2O emissions (Mosier et al., 1998; Kroeze et al., 1999). Denitrification and nitrification are the soil processes responsible for a majority of N_2O emissions from soils (Robertson and Groffman, 2007). Nitrification is the oxidation of NH_4^+ to NO_2^- and NO_3^- and it occurs primarily under aerobic conditions. Nitrous oxide is emitted during nitrification when the intermediate hydroxylamine is chemically degraded and when nitrifying bacteria reduce NO_2^- to N_2O. Denitrification is the dissimilatory reduction of NO_3^- and NO_2^- to gaseous N oxides (NO_x, N_2O, N_2) under anaerobic conditions.

Since denitrification is dependent on the primary products of nitrification (NO_2^- and NO_3^-) the two processes are often coupled; however, aerobic conditions inhibit denitrification and anaerobic conditions inhibit nitrification. Thus co-occurrence of the two processes requires temporal or spatial heterogeneity in soil O_2 concentrations. Variation in soil O_2 concentrations is primarily caused by diffusive constraints and rates of O_2 consumption (Myrold and Tiedje, 1985). Soil O_2 diffusion is a function of water-filled pore space (WPFS), bulk density, soil texture, and O_2 concentration gradients, whereas heterogeneity in soil O_2 consumption is controlled by the location and respiration rates of soil organisms.

The importance of spatial and temporal heterogeneity in soil O_2 concentrations to N_2O emissions has stimulated much work on the spatial distribution of aerobic and anaerobic soil microsites and the associated nitrifier and denitrifier communities. Parkin (1987) for example, demonstrated that a large proportion of the denitrification activity occurring in soils may be attributed to relatively rare microsites of highly concentrated labile organic matter, such as decomposing plant litter or insects. Such resource heterogeneity may help to explain the spatially structured nitrifier communities in agricultural soils as well (Grundmann and Debouzie, 2000). Using microelectrode sensors, several researchers have demonstrated resource gradients of the products and reactants of nitrification and denitrification (e.g., O_2, NO_3^-, NO_2^-, and NH_4^+)

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surrounding patches of manure (Meyer et al., 2002) and decomposing plant litter (Nielsen and Revsbech, 1998) as well as within soil aggregates (Sexstone et al., 1985).

The incorporation of plant litter into soil is a potentially important source of microbe-scale resource heterogeneity in many terrestrial ecosystems. However, as Magid et al. (2006) point out, most experimental studies of litter decomposition and its influence on N_2O fluxes have considered only uniformly distributed litter. The few exceptions have considered layered litter, and the results from these studies are mixed (Parr and Reuszer, 1959; Breland, 1994; Wang and Bakken, 1997; Bonkowski et al., 2000; Ambus et al., 2001; Magid et al., 2006). For example, Breland (1994) compared the decomposition of red clover (*Trifolium pratense* L.) shoots when uniformly distributed versus when placed in a single horizontal layer (a typical result of moldboard tillage) in a loamy soil. The layered litter initially decomposed more rapidly than the uniformly distributed. Breland postulated that the difference in litter to soil 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 versus the layered. 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 when layered. By following N mineralization in response to these two spatial distributions, 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 maize 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; Christensen et al., 2007). 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 in N mineralization from the litter. They hypothesized 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 1 mm litter versus coarsely chopped litter.

Three main hypotheses have been forwarded to explain how organic matter aggregation may alter soil microbial activity and thus CO_2 and N_2O emissions: (1) resource density-dependent expansion of trophic levels to include soil microfaunal grazers (Clarholm, 1985); (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). None have been tested in non-layered systems more typical of both natural and agricultural ecosystems nor across a range of aggregation intensity, and thus it is unclear how or why aggregation might affect decomposition and N_2O flux under realistic field conditions.

In this study we 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 and soil moisture 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.

2. Materials and methods

2.1. Experiment 1: litter aggregation and soil moisture

We performed a 2×4 factorial experiment laid out in a randomized complete block design (RCBD) with five replicates to address the question of how litter aggregation and soil moisture content affect decomposition and N_2O emissions. The treatments consisted of two levels of soil moisture (50% or 80% WFPS) and four levels of plant litter aggregation (1, 3, or 9 patches or uniform distribution). 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), which includes both Kalamazoo (fine-loamy, mixed, mesic Typic Hapludalfs) and Oshtemo (coarse-loamy, mixed mesic Typic Hapluadalfs) series. 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 g N kg^{-1}, and a pH of 5.5; similarly 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 g N kg^{-1} with a pH of 5.7 (Crum and Collins, 1995). 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 1 M KCl each to determine inorganic N concentration (Sollins et al., 1999), which 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 shoots for 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. At harvest, the shoots were cut at the sand surface, dried for 4–5 days at 55 °C, milled to pass a 10 mm screen with a Wiley mill and then ground in a Cyclotec® 1093 sample mill to pass a 1 mm screen. Eight subsamples of 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 $litter^{-1}$, providing a C:N ratio of 12.9.

2.1.1. Incubation setup

We conducted litter incubations in 2.6 L (15 cm high) 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, 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 0.08 mm low-density polyethylene

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 temperatures were recorded with a digital thermometer placed in the center of the containers; temperatures averaged 22.7 ± 1.0 °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 1 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 (Fig. 1). We constructed the patches of litter by first placing 250–450 mL of soil mix into each container, pressing a template for the litter placement into the soil and then adding the balance of the soil mix around the template so that there was 850 mL of soil in each container. The template was a 14-cm 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 initial soil was added to the containers in two steps to insure that soil was surrounding the template cylinders and not inside the cylinders as the different patch sizes required different maximum depths to achieve the same median depth in the soil. The litter was then placed into the syringe cylinders and topped off with soil. We pushed the plungers into the cylinders as the template was raised so that the litter remained in the soil as individual column-shaped patches just below the soil surface. An additional 400 mL of soil (25 mm) 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 constant mass (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.

2.1.2. 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 35–45 min with lids to allow gases to accumulate in the container headspace above the soil to be sampled (Holland et al., 1999). Gas samples were collected four times with 10 12 min between samplings. 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) with an electron capture detector (Hewlett Packard 5890 Series II, Rolling Meadows, IL, USA). An infrared gas absorption analyzer (LI-Cor 820) in series with the GC was used to determine CO_2 concentrations. We regressed the sample CO_2 and N_2O concentrations with a linear model against sampling time to determine the gas flux rate.

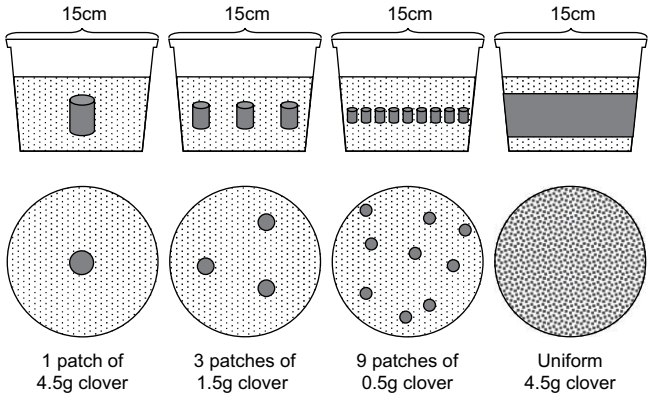

Fig. 1. Schematic depiction of spatial treatment layout of the litter aggregation gradient. The top row shows a vertical profile of the litter distribution placement in the 2.6-L containers. The bottom row of depicts a top-down view of the horizontal distribution of the litter.

2.2. 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 soil, litter, and incubation design of Experiment 1 in a 2×2 RCBD with four replicates for a total of 16 experimental units. The litter distribution treatment consisted of the uniform litter distribution and the 3-patch distribution only. The particle size treatment was composed of 1-mm and 5-mm 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 averaged 23.3 ± 1.4 °C.

2.3. Experiment 3: litter aggregation with growing plants

To assess the influence of litter aggregation on N_2O fluxes in the presence of growing plants, we scaled up the design used in Experiment 1 to accommodate a growing maize plant in 50-L containers (mesocosms) that were placed in the field. The experiment was conducted on the KBS-LTER site using a similar soil mixture as Experiments 1 and 2. We used a RCBD with 4 replicates and 5 amendment treatments: 4 litter distributions (8, 24, and 72 patches and uniform) and a fifth no-litter plus inorganic N fertilizer control treatment (control). Each of the 20 mesocosms was composed of a 50-L black plastic container filled with the soil mix, amendment, and a single maize plant (Pioneer® 35Y54). To limit the plant nutrient treatment effects to N limitations alone, we applied 250 mL of nutrient solution containing 0.5 g P, 1.25 g K, 0.25 g S-SO_4, 0.27 g Ca, and 0.07 g Mg to the soil surface of each mesocosm when the plants had reached the V-6 growth stage (6 July 2006). In addition, the control treatment received 1 L of solution containing 1.6 g N as $NaNO_3$ (equivalent to 10.7 g N m^{-2}) and litter treated mesocosms received 1 L of RO (reverse osmosis) water. Supplemental watering (2 L mesocosm^{-1}) was conducted on 13 and 22 August.

2.3.1. Litter application

We distributed 1 mm red clover litter into eight 4.69-g patches, 24 1.56-g patches, 72 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 maize, no patches were placed within the center 15 cm of the container (Fig. 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 10 cm below the soil surface. Assembly lasted from 22 to 24 May 2006, during which no precipitation was allowed into the mesocosms. The template was similar to that used in Experiments 1 and 2. The uniform treatment was constructed by placing 36 L of soil mix into 50-L mesocosms, adding a mixture of 37.5 g of litter and 4 L of soil uniformly to the entire soil surface except for the center 15 cm of the mesocosm, and then adding 10 L of soil mix on top of the litter (Fig. 2). The mesocosms were then placed in holes in the field such that the soil surface inside and outside of the mesocosms was similar. On the same day, the same variety of maize was planted by hand around each mesocosm at a standard agronomic density (60,000 plants ha^{-1}) for this region.

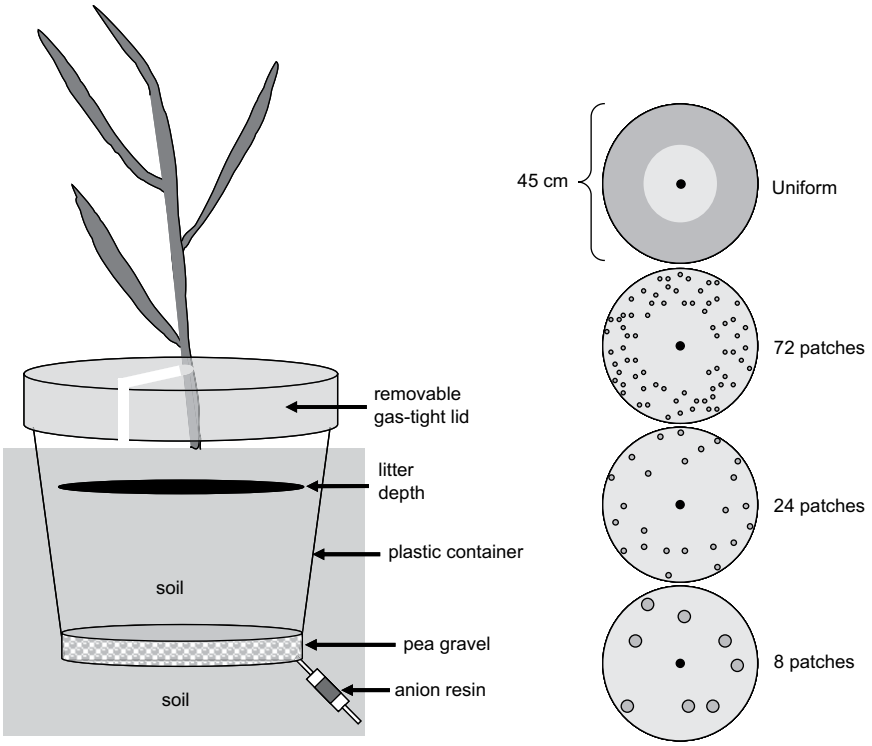

Fig. 2. Schematic depiction of the spatial litter distribution treatments conducted in 50-L mesocosms used in the field experiment. Mesocosms were set into holes in a field and surrounded by maize plants. The left panel contains a vertical-cut view of the container components. The right panel is a top-down view illustrating the spatial distribution of the litter treatments.

2.3.2. Soil surface N_2O flux

We used removable static chambers to measure soil surface N_2O flux (Holland et al., 1999). Each chamber lid was sealed around the outside of the container and around the plant stem (Fig. 2). The chamber lids were modified from 120-L high density polyethylene refuse containers and lids with a 60 mm wide slit removed from the edge to the center of the chamber to accommodate the plant stem. Latex sheeting was secured to the plant stem and chamber lid to complete an airtight seal. Deployment of the chamber lid required ~3 min and remained on the container for a maximum of 70 min during sampling. We used a similar gas sampling and analysis procedure as Experiments 1 and 2.

2.4. Statistical analysis

All flux data were natural log transformed to meet the analysis of variance (ANOVA) assumption of homogeneity of variances. Each experiment was analyzed as a repeated measure ANOVA. Experiment 1 was analyzed as a full factorial design with block, moisture, litter distribution, and the main effect interactions as independent fixed variables and sampling date as the repeated random variable. The second experiment was analyzed with block, litter distribution, particle size, and the main effect interaction as the independent fixed variables and sampling date as the repeated measure. For Experiment 3, block, litter distribution, and the main effect interactions were the fixed effects and sampling date was 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 all three 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 System (Littell et al., 2005). Differences were considered significant at the $\alpha = 0.05$ level for all ANOVAs.

3. Results

3.1. Experiment 1: litter aggregation and soil moisture

The decomposition rate of red clover litter (quantified as CO_2 flux) was dependent on its spatial aggregation and soil WFPS (Fig. 3). Litter decomposition was slower in the wet soil (80% WFPS) than in the moist soil (50% WPPS); however, the differences in decomposition rate between the two soil moisture treatments decreased with incubation time.

At 50% WFPS, litter decomposition varied through time with litter aggregation treatment. The decomposition rate of the uniformly distributed litter was greatest at the first sampling and then declined exponentially with incubation time as expected. In contrast, the aggregated litter (1, 3, and 9 patches) did not achieve maximum decomposition rate until incubation day 5. This initial delay in the decomposition of the aggregated litter led to significant differences in the decomposition rate between uniformly distributed and aggregated litter for the first 10 days of the incubation (Fig. 3). 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 fastest, 9-patch and uniform distributions were decomposing at an intermediate rate, and the single large patch treatment was decomposing at the slowest rate.

In the wet soil treatment (80% WFPS), uniformly distributed litter consistently decomposed more rapidly than aggregated litter (Fig. 3). Among the aggregated litter treatments, litter aggregated into a single patch and three patches decomposed at a greater rate on average (4.5 ± 0.3 and 5.4 ± 0.6 mg C m^{-2} h^{-1}, respectively) than the 9-patch distribution (3.2 ± 0.5 mg C m^{-2} h^{-1}).

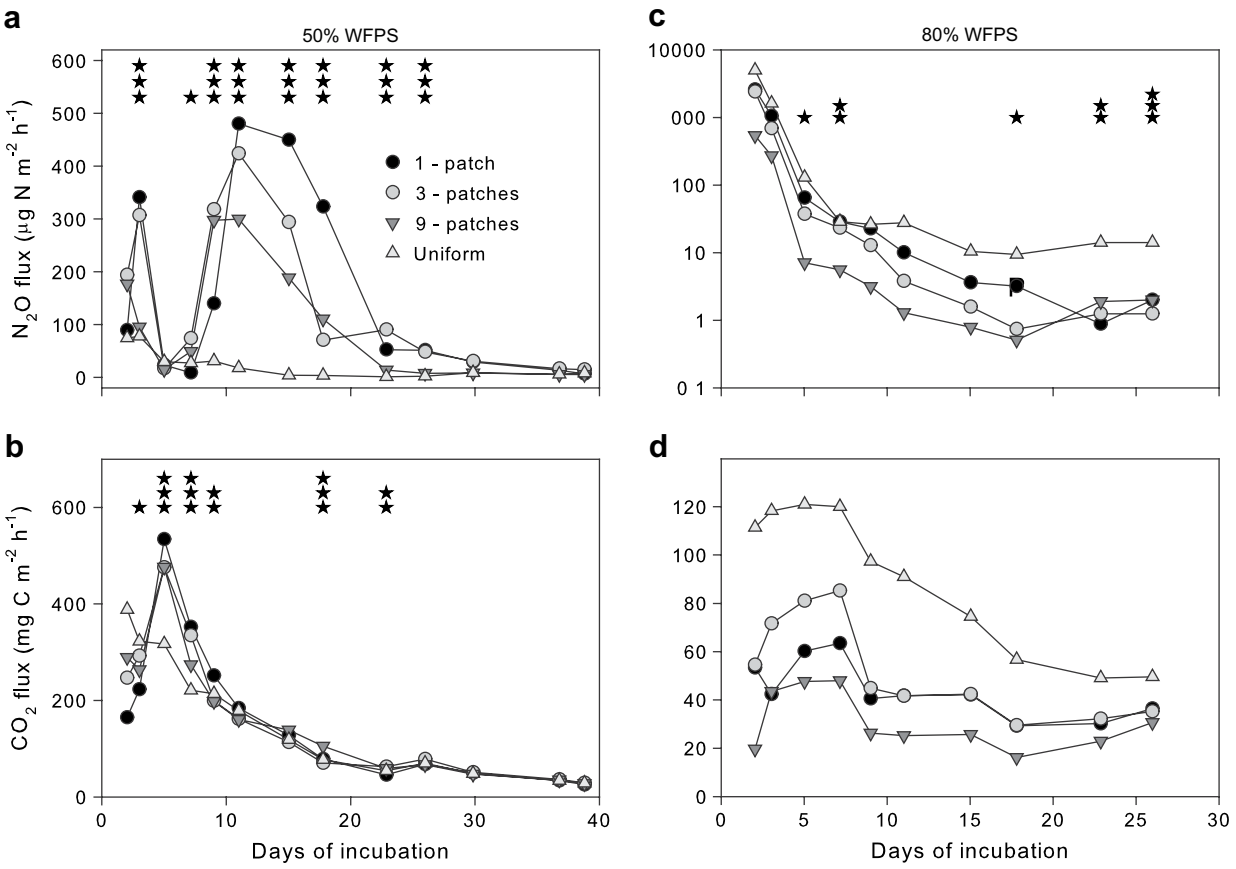

Fig. 3. Litter aggregation and soil moisture content influence on CO_2 and N_2O flux. (a,c) N_2O flux from litter aggregation as influenced by 50% and 80% WFPS, respectively; (b,d) CO_2 flux from litter aggregation as influenced by 50% and 80% WFPS, respectively. ANOVA results comparing the mean of the aggregated litter treatments to the uniformly distributed litter are presented across the top of each graph. Statistical significance at $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ for the contrasts. In (d) the repeated measures ANOVA indicates that the CO_2 flux was significantly greater ($P < 0.05$) in the uniform distribution than the mean of the aggregated litter throughout the entire incubation. Note the differences in scales between the left and right panels.

The effect of litter aggregation on N_2O fluxes varied with soil moisture (% WFPS) and sampling date (Figs. 3 and 4). The 80% WFPS treatment produced a wider range of N_2O fluxes (0.5–5000 μg N m^{-2} h^{-1}) than the 50% WFPS treatment (0.9–480 μg N m^{-2} h^{-1}). Highest N_2O flux rates were found between 10 and 15 days into the incubation for the 50% WFPS treatment whereas for the 80% WFPS treatment the first sampling date had the highest N_2O rates. In both soil moisture treatments, N_2O flux response to litter aggregation was affected by time of sampling.

At 50% WFPS, N_2O fluxes from the uniform litter distribution generally decreased with the progression of the incubation, whereas the aggregated litter produced more variable N_2O fluxes (Fig. 3). Nitrous oxide fluxes from the uniform litter distribution were lower than or indistinguishable from the patchy litter distribution treatments throughout the incubation. Statistical differences occurred among the litter aggregation treatments at 2 days and 7–26 days into the incubation under 50% WFPS (Fig. 3). Nitrous oxide flux from the three patchy litter distributions was 259% greater on average than the uniform litter distribution at days 3, 5, and 9–26 of the incubation at 50% WFPS. The overall mean N_2O flux during the incubation at 50% WFPS was greatest when the litter was aggregated into a single patch (157 ± 26μg N m^{-2} h^{-1}) and 3 patches (143 ± 22 μg N m^{-2} h^{-1}), intermediate when aggregated into 9 patches (94 ± 15 μg N m^{-2} h^{-1}), and least when uniformly distributed (20 ± 4 μg N m^{-2} h^{-1}).

At 80% WFPS, N_2O fluxes decreased with time of incubation (Fig. 3). At this soil moisture, the uniform distribution emitted more N_2O on 5 of the 10 sampling dates (5, 7, 18, 23, and 26 days of incubation) than the mean of the patchy litter distributions and was similar across the other 5 sampling dates.

3.2. Experiment 2: litter particle size and aggregation

Litter decomposition rate varied with aggregation treatment and litter particle size throughout the 18-day incubation (Fig. 4). Within 20 h of adding water to these containers the decomposition rate of the uniformly distributed litter was higher than the patchy distributions. By the fourth day of incubation this trend was reversed with the patchy litter distribution decomposing more rapidly than the uniform litter distribution. This pattern continued through day 14 of the incubation with the exception of no differences on day 7. On the last two sampling dates litter distribution did not affect the decomposition rate. The litter particle size affected decomposition on four dates. On day 6, the coarse litter was decomposing more rapidly than the fine litter; however, during the last three sampling dates this trend was reversed such that the fine litter was decomposing more rapidly than the coarse litter.

The patterns of N_2O flux in response to litter aggregation and particle size were more complex than were CO_2 flux patterns (Table 1). On 7 of the 9 sampling dates, there were significant interactions between the two main factors, particle size and litter aggregation. Initially, N_2O fluxes of the uniformly distributed coarse litter were higher than that of the uniformly distributed fine particles and the N_2O fluxes from the patchy distributed litter were intermediate. By day 4 of the incubation the patches of litter were emitting more N_2O than the uniformly distributed litter. During the

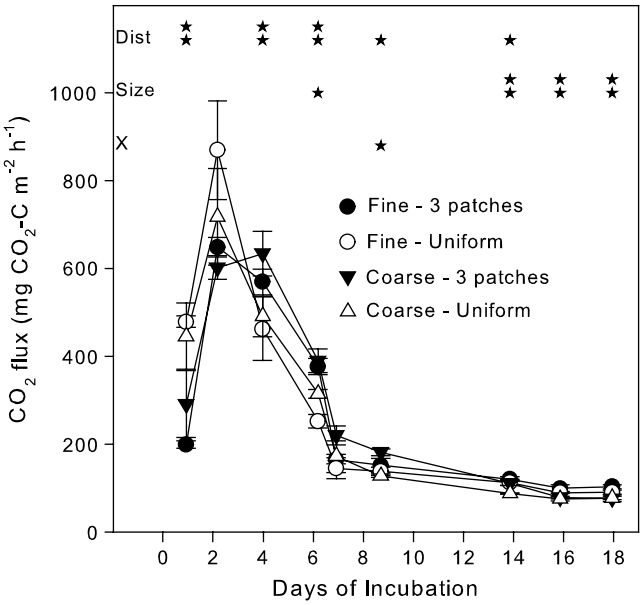

Fig. 4. Carbon dioxide flux response to litter aggregation and litter particle size during an 18-day incubation. ANOVA results are presented across the top of each graph. Statistical significance at $^{*}P < 0.05$ and $^{**}P < 0.01$ levels for the two main effects, Distribution (Dist) and Particle Size (Size), and their interaction term (*X*). Error bars indicate one standard error.

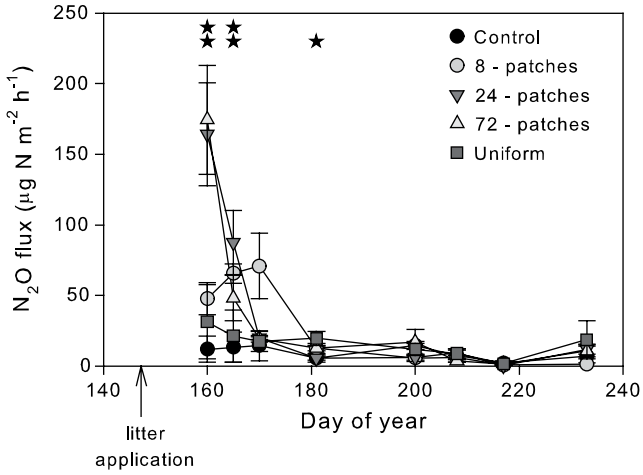

Fig. 5. Nitrous oxide flux by day of year (DOY) from litter aggregation with field-grown maize plants in 50-L mesocosms. ANOVA results are presented across the top of the graph. Statistical significance at the $^{*}P < 0.05$ and $^{**}P < 0.01$, for the contrast between aggregated and uniformly distributed litter. The error bars indicate one standard error.

last four samplings N_2O fluxes were consistently greatest from the fine aggregated litter and among the lowest from the fine uniform litter distribution, whereas N_2O fluxes of the coarse litter treatments were similar to or greater than the fine uniform distribution.

3.3. Experiment 3: litter aggregation with growing plants

Under field conditions with growing maize plants, N_2O fluxes were highest at the beginning of the sampling period and varied in response to litter distribution (Fig. 5). Fifteen and 20 days after litter application on day of year (DOY) 160 and 165, N_2O emissions were on average 4.1 and 3.2 times greater, respectively, from aggregated litter than uniformly distributed litter (Fig. 5). In contrast, on DOY 181 N_2O fluxes were 1.9 times greater from uniformly distributed litter than aggregated litter. Across the entire sampling period, the three litter aggregation treatments (8, 24, and 72 patches) produced similar time-weighted mean N_2O fluxes (18.1 ± 1.7, 18.6 ± 2.4, and 17.3 ± 1.2 µg N_2O-N m^{-2} h^{-1}, respectively) which were greater than from the uniformly distributed litter (8.8 ± 1.5 µg N_2O-N m^{-2} h^{-1}) and the no-litter control (5.9 ± 0.3 µg N_2O-N m^{-2} h^{-1}).

4. Discussion

4.1. Influence of litter aggregation on decomposition

We found that aggregation of red clover litter in soil delayed the peak litter decomposition rate by 4–5 days compared to uniformly distributed litter (Fig. 3). In comparison Magid et al. (2006) found that layering maize litter or sheep feces in soil delayed and dampened their decomposition rates relative to uniformly distributed litter by 11–16 days. This delay corresponded to more total inorganic N and less microbial biomass N in the system with layered litter than with uniformly distributed litter. From this, Magid et al. (2006) suggested that decomposition of the layered litter was more limited by inorganic N availability than distributed litter because the diffusive flux of NO_3^- into the layered litter is only slightly greater (due to sharper NO_3^- gradient) than into the distributed litter particles, while simultaneously the microbial NO_3^- immobilization rate per volume of soil in the layered litter is many fold greater than in the distributed litter particles. In other words, the NO_3^- supply rate is similar in both layered and uniform litter distributions; however, the microsite demand for NO_3^- is much greater in layered than in uniformly distributed litter.

The maize litter used by Magid et al. (2006) was relatively N-poor and C-rich (C:N of 53) compared to our red clover litter (C:N of 12.9); thus, the red clover decomposition was not as likely to be limited by inorganic N availability as the maize litter. However, on

Table 1
Analysis of variance and means of N_2O flux rate in response to litter particle size and aggregation treatment by sampling date. Means with multiple comparisons adjusted by Tukey–Kramer procedure

Source of variance	Particle size	Aggregation	Aggregation × size	Treatment means (µg N m^{-2} h^{-1})[b]			
d of incubation	*P>F*			Fine		Coarse	
	1,9[a]	1,9	1,9	Uniform	Three patches	Uniform	Three patches
0.9	0.0348	0.7589	0.0404	41.8 b	67.4 ab	135.3 a	69.9 ab
2.2	0.3355	0.1734	0.6971	124.5 ns	168.9 ns	90.3 ns	137.6 ns
4.0	0.0008	0.0001	0.0090	12.2 c	170.0 a	58.9 b	219.7 a
6.2	0.3546	0.0001	0.0001	7.2 c	77.0 a	30.0 b	29.5 b
6.9	0.3953	0.3515	0.0586	6.3 ns	32.6 ns	22.7 ns	14.9 ns
8.7	0.9208	0.0001	0.0001	5.9 c	48.8 a	15.6 b	23.9 b
13.9	0.0004	0.0001	0.0012	1.9 c	373.0 a	1.2 c	28.9 b
15.9	0.0001	0.0001	0.0001	2.6 c	232.1 a	4.9 c	12.0 b
17.9	0.0001	0.0001	0.0001	0.3 c	153.1 a	5.5 b	7.6 b

[a] Indicates the numerator and denominator degrees of freedom, respectively.
[b] Different letters following means within rows indicate significant differences ($\alpha = 0.05$).

day 5 of our incubation we saw a suggestion of limited NO_3^- availability as indicated by the sharp drop in N_2O emissions that coincide with the maximum rate of CO_2 flux from the aggregated litter (Fig. 3). The high CO_2 flux indicates an increased demand for terminal electron acceptors and thus the concurrent reduction in N_2O gas flux likely indicates a more complete reduction of N_2O to N_2 or NO_3^- depletion due to microbial assimilation or both. Despite this temporary NO_3^- limitation around day 5, the initial inhibition of litter decomposition at the beginning of the incubation is not likely to be due to limited inorganic N, because N_2O fluxes were relatively high at the onset of the incubation at both 50 and 80% WFPS.

An alternative explanation for the delay in peak litter decomposition rate of the aggregated litter is that soil O_2 availability may have been more limiting in the aggregated litter than in the uniformly distributed litter. Anaerobic conditions and processes (e.g., denitrification) have been observed in otherwise aerobic bulk soils (Robertson and Tiedje, 1987), soil aggregates (Sexstone et al., 1985), rhizospheres (Smith and Tiedje, 1979), and surrounding decomposing organic matter (e.g., Parkin, 1987). We incubated the red clover litter at two soil moisture conditions (50 and 80% WFPS) as a proxy for understanding how soil O_2 (Bollmann and Conrad, 1998) affects decomposition processes in the aggregated and uniformly distributed litter (Fig. 3). The initial delay in decomposition rate of the aggregated litter observed at 50% WFPS was not evident at 80% WFPS. Furthermore, at 80% WFPS the uniformly distributed litter decomposed faster than the aggregated litter throughout the incubation period. These results likely indicate that the mechanisms causing the differences in litter decomposition between distributed and aggregated litter at 50% WFPS were less important at 80% WFPS. We suggest that the difference in O_2 concentration between the aggregated and the distributed litter at 50% WFPS caused the initial delay in the aggregated litter decomposition, but at 80% WFPS the O_2 concentration differences between the aggregated and distributed litter were much smaller and O_2 limitation was similar regardless of litter distribution. This suggestion is consistent with the work of Christensen et al. (1990) on the influence of patchy distributions of labile organic matter on denitrification rates.

The litter decomposition rates at 80% WFPS among the aggregated litter (1, 3, and 9 patches) do not show a linear decrease in decomposition rate with increasing aggregation (Fig. 3) as expected if O_2 diffusion were the only factor limiting decomposition. This may indicate a combination of factors limiting litter decomposition when aggregated under wet conditions; however, it is worth noting that the difference in decomposition rates among the three aggregation treatments is small relative to the differences between the aggregated and uniformly distributed litter. This nonlinear trend of the influence of litter aggregation intensity on decomposition rates under 80% WFPS may indicate greater complexity of microbial process rate regulation, but does not change the conclusion that soil O_2 availability was the most important factor causing the temporary delay in decomposition of aggregated litter at 50% WFPS.

Breland (1994) observed the opposite influence of litter aggregation on decomposition than our or Magid et al.'s (2006) data. In Breland's experiments, layering red clover shoots in the soil stimulated the cumulative CO_2 flux relative to uniformly distributed red clover litter. Breland attributed this dampened decomposition rate to the greater litter particle-to-mineral surface contact that results when the litter is distributed versus layered. He hypothesized that litter particle contact with mineral surfaces offers a form of physical and chemical protection from microbial degradation. We tested this hypothesis in Experiments 1 and 2 by distributing litter across an aggregation gradient from evenly distributed to highly aggregated in order to vary the contact between the litter and mineral surfaces. Our results suggest that the degree of soil–litter contact did not influence the litter decomposition rate; we found similar decomposition patterns across our aggregated litter distributions. However, soil texture is likely an important difference between our experiment and Breland's. Our soil mixture was half loam and half sand, whereas Breland used a loamy soil. Loamy soils contain more clay and thus more mineral surface area per unit of mass than sandy soils and thus may provide more physical protection of fine litter particles from microbial degradation.

Particle size is often an important control on litter decomposition rate because smaller particles are thought to provide less structural resistance to microbial degradation than larger more intact plant litter (Rovira and Vallejo, 2002) and influence microbivorous microfauna access to microbial prey (Vestergaard et al., 2001). We found that red clover shoots decompose at a similar rate for the first 2 weeks of incubation regardless of particle size (Fig. 4). However, after the first 2 weeks the fine red clover particles began to decompose at a faster rate than the coarser particles. This suggests that during the first 2 weeks soluble organic compounds contained in the litter were the primary source of microbial energy. If this was the case then the aggregation effect of delayed peak decomposition is mostly due to the inhibition of the oxidation of the soluble litter components, and as those soluble organics are depleted the physical protection of larger particles from microbial degradation becomes more important (Moorhead and Sinsabaugh, 2006).

4.2. Nitrous oxide response to litter aggregation

The incorporation of crop and cover crop litter into soil as a means to improve soil fertility and quality is a common practice throughout the world (Kumar and Goh, 2000), and it appears to result in a heterogeneous distribution of plant litter in tilled soil (Staricka et al., 1991). Nitrous oxide emissions from temperate row-crop production systems are similar when legume cover crops or inorganic fertilizers are the main source of N fertility (e.g., Robertson et al., 2000); however, N_2O flux rates can vary spatially by several fold within a homogeneously managed plot (Rover et al., 1999). The results of our laboratory incubation experiments indicate that under moist soil conditions (50% WFPS), the greater the intensity of red clover litter aggregation the greater the N_2O emissions (Fig. 3 and Table 1). This result is in contrast to Ambus et al. (2001), who found that layered alfalfa and wheat litter depressed N_2O emissions by 1.6 and 6.5 times, respectively, relative to uniform litter. They hypothesized that the N mineralization from these two litters was more limited by inorganic N availability when layered than when distributed, and that this lack of inorganic N caused greater N_2O reduction to N_2 and greater microbial immobilization. Depressed NO_3^- availability in our aggregated litter treatment likely caused the sharp decline in N_2O emissions around days 5–7 (Fig. 3), although the effect was temporary and at no point during the incubation was N_2O flux greater from the uniformly distributed than from any aggregated treatment (Fig. 3), regardless of litter particle size (Table 1).

In contrast, under near-saturated soil moisture conditions (80% WFPS) the uniformly distributed litter treatment consistently emitted more N_2O than any of the aggregated treatments (Fig. 3). This effect is likely the result of two co-occurring processes: (1) greater O_2 consumption in the uniform litter as indicated by greater CO_2 emission rate, and (2) more denitrifier access to NO_3^- diffusing from the surrounding soil. As soil moisture increases, the flux of O_2 in the soil is inhibited, whereas the movement of NO_3^- is promoted because more water means less gaseous pore space through which O_2 can move and a less tortuous path for NO_3^- diffusion (Myrold and Tiedje, 1985).

The influence of litter aggregation or litter layering in soil on N_2O emissions has not been extensively explored under field conditions, where the primary drivers of N_2O emissions can vary widely (e.g., soil moisture, O_2, labile C, and habitat and organismal

complexity). The patterns of N_2O emissions from our field mesocosms experiment generally agree with the laboratory incubation experiments (Figs. 3 and 5). The N_2O emissions from field Experiment 3 (6–19 μg N_2O-N m^{-2} h^{-1}) were similar to those of the laboratory incubation studies (Experiments 1 and 2) 15–20 days after litter application and to the growing season average fluxes of the nearby KBS-LTER cropping systems experiment (~13 μg N_2O-N m^{-2} h^{-1}) (Grandy et al., 2006).

For the purpose of estimating agriculture's contribution to global greenhouse gas emissions, N_2O emissions are assumed to be a constant (1.25%) proportion of the total N added to soils regardless of the form applied (e.g., inorganic or organic) (IPCC, 2007). Our results along with those of Ambus et al. (2001) suggest that the same quantity of litter-N can markedly influence N_2O emission rates depending only on its spatial distribution within the soil. Current agricultural practices result in highly aggregated litter placement following soil tillage. Our results suggest that this maximizes the potential N_2O flux, whereas distributing litter more evenly or in layers might substantially reduce fluxes. Further field experiments are warranted.

4.3. Conclusions

The aggregation of red clover litter in soil temporarily delayed peak decomposition rates under moist soil conditions (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 promoted N_2O emission in both laboratory incubations and field mesocosms, and emissions increased with litter aggregation intensity; the most aggregated litter treatment in our experiments emitted 8 times more N_2O than treatments with uniformly distributed litter. These observations have important implications for how we estimate N_2O emissions for greenhouse gas inventories from cropping systems and design N_2O mitigation strategies.

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