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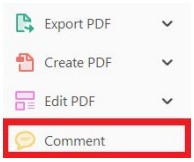

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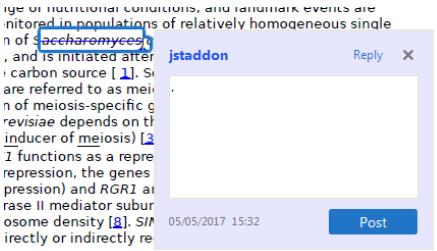

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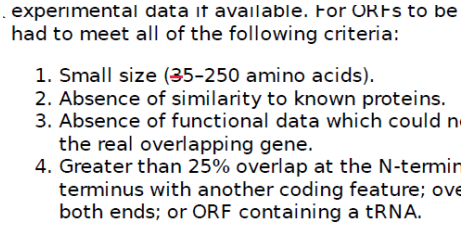

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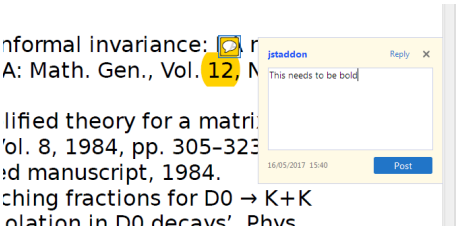

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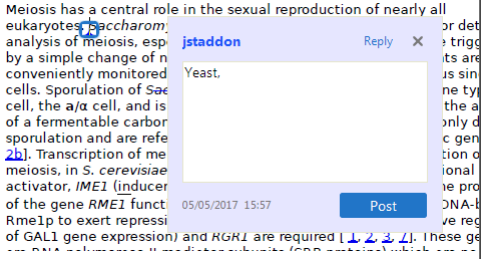

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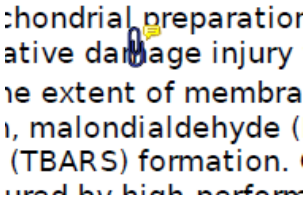

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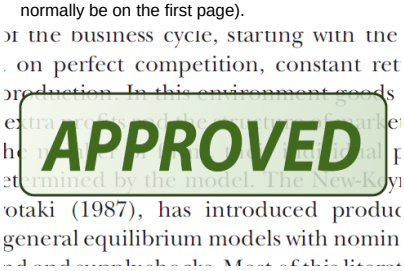

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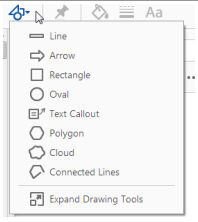

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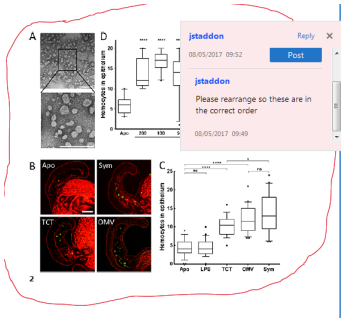

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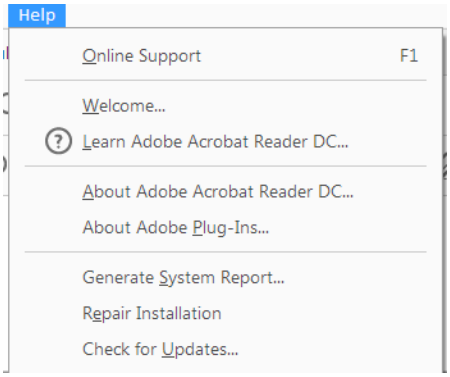

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Of macropores and tillage: influence of biomass incorporation on cover crop decomposition and soil respiration

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Abstract

Carbon sequestration in agricultural soils may help to reduce global greenhouse gas concentrations, but building up soil carbon levels requires accumulating organic matter faster than it is lost via heterotrophic respiration. Using field and laboratory studies, this study sought to elucidate how tillage, the below-ground incorporation of cover crop residue, and soil macroporosity affect soil respiration and residue decomposition rates. In the field, residue from a cover crop mixture of barley (*Hordeum vulgare*) and crimson clover (*Trifolium incarnatum*) was placed into litter bags that were left on the surface versus incorporated into the soil at three depths (4, 8 or 12 cm), while the laboratory study compared surface-placed versus incorporated litter (8 cm depth). To assess tillage effects on cover crop decomposition, the field study simulated no-till and conventional tillage treatments, while the laboratory and field studies both included treatments in which artificial soil macropores were created. The field study showed that conventional tillage and the presence of macropores enhanced soil respiration, while in the laboratory study, incorporating cover crop residue resulted in higher soil respiration and faster litter decomposition rates. Additionally, the laboratory measurements showed that macropores increased soil respiration in wet conditions, likely by enhancing oxygen diffusion. Thus, organic matter incorporation and macropores may represent important factors that affect soil respiration and carbon dynamics.

Keywords: Soil respiration, CO_2, carbon dioxide, macropores, tillage, cover crops

Introduction

Globally, row crop agriculture covers 1.7 billion hectares and represents a soil carbon stock of ~170 Pg (Paustian *et al.*, 1998). Carbon sequestration in agricultural soils has the potential to reduce global greenhouse gas concentrations, but building up carbon reserves requires accumulating organic matter from crop residue faster than those materials are lost via heterotrophic respiration. Conservation agricultural practices, such as reduced/no tillage and the inclusion of cover crops into rotations, have the potential to annually sequester 0.4–1.2 Pg of carbon (Lal, 2004). However, individual studies have found substantial variability in the effectiveness of conservation agriculture for retaining soil carbon, with many of the controlling mechanisms not fully understood (Govaerts *et al.*, 2009).

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In agricultural systems, heterotrophic soil respiration is affected by many biological, chemical and environmental factors, including soil carbon lability (Gu *et al.*, 2004) and biomass C : N ratio. Residue possessing low C : N ratios (i.e. <20 : 1) typically decomposes faster than residue with high ratios (Aulakh *et al.*, 1991; Coppens *et al.*, 2007). Soil wetness and temperature also influence microbial activity and respiration (Davidson *et al.*, 1998; Yonemura *et al.*, 2014). Soil respiration in well-drained soils often peaks when the water-filled pore space (WFPS) is ~60% of saturated (Groffman & Tiedje, 1991), as this value represents an optimal condition where microbes are fully functioning (i.e. no substrate limitations) yet soil gas diffusion rates are still high enough to allow O_2 and CO_2 exchange (Skopp *et al.*, 1990). Tillage can temporarily boost respiration rates by exposing organic matter to microbes and facilitating aerobic environments in the tilled zones (Hendrix *et al.*, 1988). However, soil respiration tends to decrease within days to weeks after tillage, as organic residues are consumed and soil particles re-orient themselves into denser arrangements that

may inhibit gas exchange (Reicosky, 1997). Over annual time-scales, soil respiration from no-tilled and conventionally tilled soils can be nearly identical (Franzluebbers *et al.*, 1995), although other studies have shown more variable long-term soil respiration patterns, potentially due to tillage-induced differences in soil temperature and water content (MacDonald *et al.*, 2010; Morell *et al.*, 2011; Yonemura *et al.*, 2014). Tillage also affects soil structure and porosity, with conventional tillage typically resulting in diminishment of soil structure, development of subsurface compaction layers, and loss of aggregate stability (Bronick & Lal, 2005). These factors can all restrict water and air movement (Pagliai *et al.*, 2004).

Cover crops may potentially increase soil organic matter levels when added to rotations (McDaniel *et al.*, 2014), although biomass accumulation will be affected by planting and termination dates, type of cover crop and climate (Brandsæter *et al.*, 2008). While most studies of cover crop inclusion in no-till systems have found that organic matter accumulation primarily occurs in the upper 5–10 cm of soil (Fronning *et al.*, 2008; Nascente *et al.*, 2013), more general studies of crop residue incorporation have demonstrated variable effects for soil respiration and residue decomposition. For example, Kainiemi *et al.* (2015) showed that biomass incorporation decreased soil respiration rates compared to leaving the residue on the surface, whereas Coppens *et al.* (2007) observed faster decomposition for incorporated litter compared to surface mulches, with soil moisture and nitrogen availability seen as primary controls. Rottmann *et al.* (2010) showed that heterotrophic soil respiration increased when plant litter was incorporated at 0–5 cm compared to 15–20 cm depths. Still, the specific role of cover crop biomass incorporation on subsequent microbial respiration and decomposition rates remains unclear.

Another potential factor influencing soil respiration is macroporosity. Macropores, often defined as any pore larger than 0.1 cm (Luxmoore, 1981), can arise from biopores (e.g. root channels, wormholes), desiccation cracks or structural features. Macropores can drive the preferential flow of water (van Schaik *et al.*, 2013; Stewart *et al.*, 2015; Angermann *et al.*, 2016) and solutes (Bronswijk *et al.*, 1995; van der Salm *et al.*, 2012), enhance water vapour exchange (Weisbrod *et al.*, 2009), and increase effective soil gas diffusivity (Schaefer *et al.*, 1997; Kristensen *et al.*, 2010). Still, the specific role of macropores in mediating soil respiration and greenhouse gas generation remains uncertain (Blagodatsky & Smith, 2012). Many studies have determined that no-till systems, particularly those including cover crops, may increase soil macroporosity (Strudley *et al.*, 2008; Abdollahi *et al.*, 2014). Nonetheless, at least one field study showed that the conventionally tilled soils had higher macroporosity and increased greenhouse gas emissions compared to no-till soils (Mangalassery *et al.*, 2014).

In recognition of these knowledge gaps, this study had two main objectives:

1. Evaluate how different tillage practices and biomass incorporation depths affect decomposition of cover crop residues, and
2. Quantify macropore effects on soil respiration, gas exchange and plant residue decomposition.

By addressing these objectives, this study offers improved understanding of dynamic soil controls on residue decomposition and soil respiration rates.

Materials and methods

Experimental set-up and treatments

The study included a field and laboratory study. The field site was located approximately 6 km west of the Blacksburg, Virginia, in a long-term no-till corn field. The soil was a Groseclose (*Fine, mixed, semiactive, mesic Typic Hapludults*) and Poplimento (*Fine, mixed, subactive, mesic Ultic Hapludalfs*) undifferentiated group, with a silty loam texture (NRCS, 2017).

For the field portion, 60 litter bags (14 × 15 cm) were created from 0.1-cm nylon mesh (Figure 1). The cover crop litter bags were filled with 6 g of air-dried stalk/stem tissue (cut to 5 cm length) and 4 g of air-dried grain from a mixture of barley (*Hordeum vulgare*) and crimson clover (*Trifolium incarnatum*). Litter bags were installed on 20 June 2016 in a 6 × 10 m grid. Litterbags were installed at one of three depths: 4, 8 or 12 cm. For each buried litterbag, one of the following tillage treatments was applied:

1. Conventional tillage (CT). During installation, soil above the litter bag was hand-crumbled into < 1-cm-sized aggregates.
2. No tillage (NT). A 30 × 40 cm soil monolith was cut down to the desired depth, and then lifted while the litter bag was slid underneath. Soil was kept intact with minimal disturbance.
3. No tillage with artificial macropores (MP). Similar to the NT installation, litter bags were placed beneath intact monoliths. However, each monolith received nine artificial macropores (soil holes) spaced 3 cm apart (Figure 1). Macropores were created by hand-drilling the soil with a Philips screwdriver to the depth of the litterbag and were maintained using rigid paper straws (0.5 cm inner diameter).

A fourth treatment was included in which litter bags were left on the surface (S). Six litter bags were randomly assigned to the surface treatment and each combination of tillage type and incorporation depth. Litter bags were harvested after either 17 days or 36 days ($n = 3$).

CO_2 flux and litter decomposition measurements

Soil respiration [$N/L^2/T$] was quantified using an infrared CO_2 gas flux analyser (LI-COR 8100) atop a 20-cm sampling

Figure 1 (Left) field treatment with surface-applied litter bag; (centre) field treatment with artificial macropores; (right) laboratory column treatment with artificial macropores.

collar. Near-surface soil water content [L^3/L^3] was measured with a moisture probe (Decagon Devices EC-5 sensor). Chamber temperature was also recorded during each measurement. Soil respiration measurements were collected from the 36-day treatment plots on days 1, 3, 5, 8, 19, 22, 31 and 36. Cumulative CO_2 production [N/L^2] was estimated for each plot using the trapezoidal integration method, in which the average flux between two measurements was multiplied by the length of time between those measurements.

After being exhumed, litter bag residue was weighed, oven-dried at 40 °C for 48 h and reweighed. Decomposition rates [M/T] were determined as total change in residue mass divided by number of elapsed days (17 or 36). Daily precipitation [L] was measured using a flow through rain collector (Spectrum Technologies) located 3 km from the field site.

Column study

A column study was used to isolate heterotrophic contributions to soil respiration under controlled environmental conditions. Sixteen columns (20 cm diameter × 15 cm height) were packed to a dry bulk density of 1.34 g/cm^3 using 4-mm sieved soil from the field site. Final soil height was 11 cm, leaving a 4-cm headspace. Sixteen 14 × 15 cm nylon mesh litter bags were filled with 10 g of dry cover crop residue (4 g grain, 6 g stem/stalks).

Four treatments were used (n = 4):

1. Surface ~~L~~litter (SL) – Litter bag was placed on the soil surface.
2. Incorporated ~~L~~litter (IL) – Litter bag was packed at a depth of 8 cm.
3. Incorporated ~~L~~litter with ~~M~~macropores (ILM) – Similar to the IL treatment, except nine 0.5-cm-diameter artificial macropores were installed using rigid paper straws from the litterbag depth to the soil surface (Figure 1).
4. No-litter ~~C~~control (C) – ~~No-litter bag was included~~.

The experiment began on 22 July 2016. CO_2 fluxes were measured with the LI-8100 gas analyser on days 1, 2, 4, 8, 11, 13, 16, 20, 26, 27, 29, 49, 50, 56 and 61, and the trapezoidal integration method was used to determine cumulative CO_2 production [N/L^2]. After each flux measurement, columns were weighed on a balance (Scientific Products, Evanston, IL, USA). Mass of water (m_w) was calculated by subtracting the mass of the column, dry soil (m_s), and full litterbag. Gravimetric water content (w) was determined as:

$$w = \frac{m_w}{m_s} \tag{1}$$

Volumetric water content (θ) was found using the density of water (ρ_w = 1 g/cm^3) and the packed bulk density of the soil (ρ_b = 1.34 g/cm^3), by:

$$\theta = w\left(\frac{\rho_b}{\rho_w}\right) \tag{2}$$

Water-filled pore space was determined by dividing the volumetric water content by the material porosity (ϕ = 0.495 cm^3/cm^3):

$$\text{WFPS} = \frac{\theta}{\phi} \tag{3}$$

The columns were rewetted with tap water to θ = 0.38 cm^3/cm^3 on days 8 and 26, and to θ = 0.33 cm^3/

cm^3 on Day 49. Temperature was maintained at 22 ± 1 °C.

On Day 61 (20 September 2016), the columns were disassembled. The litter bags residue was oven-dried at 40 °C (48 h) to determine final mass; decomposition rates were determined as change in litter mass divided by number of elapsed days (61).

Soil respiration (f) was modelled as a function of water content for the incorporated litter treatments (ILM and IL) using a piecewise function (Skopp *et al.*, 1990):

$$f = \begin{Bmatrix} a\theta^c \\ b(\phi - \theta)^d \end{Bmatrix} \quad \begin{matrix} \theta < \theta_m \\ \theta \geq \theta_m \end{matrix} \tag{4}$$

where a, b, c and d are empirical constants and θ_m is the water content of maximum respiration.

To fit Equation 4, we set θ_m using the water content with the highest observed flux for either treatment, and constrained a to ensure that f was continuous at θ_m:

$$a = \frac{b(\phi - \theta_m)^d}{\theta_m^c} \tag{5}$$

We then used a least-squares regression between the measured and modelled fluxes to determine optimum parameter values for b, c and d.

Statistical analyses

Statistical analyses were performed using R (version 3.4.1). For the field data, a one-way analysis of variation (ANOVA) with Tukey HSD was done to compare measured CO_2 fluxes and time-integrated CO_2 production values between tillage treatments based on the three depths of incorporation (4, 8 and 12 cm). Mean daily CO_2 flux and cumulative CO_2 production values (averaged across incorporation depths) were used to compare the tillage treatments and the surface-applied litter treatment, S (one-way ANOVAs with Tukey HSD). A t-test was used to compare the 17- and 36-day residue decomposition rate for each tillage treatment and depth combination. For the column study data, mean decomposition rates were compared between treatments using a one-way ANOVA. $\alpha = 0.1$ was used to identify significance.

Results

Field study

The ~~C~~onventional ~~T~~illage (CT) treatment had the highest mean respiration rates, while the ~~S~~urface (S) treatment had the lowest (Figure 2 shows mean respiration values across all depths; individual depths are shown in Figure S1). CO_2 fluxes from the CT treatments were significantly higher than

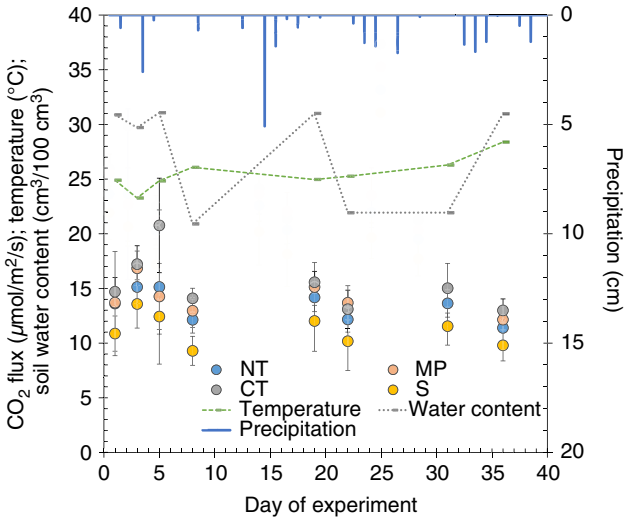

Figure 2 CO_2 fluxes (μmol/m^2/s), flux chamber temperature (°C), upper soil water content (cm^3/100 cm^3) and precipitation (cm) measured during the study. CT, conventional till; MP, no-till with artificial macropores; NT, no-till; S, surface-applied litter. Error bars indicate ±1 standard deviation.

the ~~N~~o tillage (NT) treatments for days 8 (12 cm depth; Tukey, $P = 0.04$), 19 (8 cm depth; Tukey, $P = 0.09$) and 36 (12 cm depth; Tukey, $P = 0.06$). Artificial macropores (MP) caused slightly higher mean respiration fluxes than the no-till without macropores (NT), with significant differences seen on Day 22 (12 cm depth; Tukey, $P = 0.02$).

Differences between treatments were more evident for cumulative CO_2 production (Figure 3 shows mean CO_2 production across all depths; individual depths are shown in Figure S2). CT had the highest overall CO_2 production, followed by the MP and then NT treatments (Figure 3). Surface-placed bags had significantly lower CO_2 production than the other treatments (Tukey, CT-S: $P = 0.0055$, MP-S: $P = 0.0082$; NT-S: $P = 0.075$). The CT treatment also showed significantly higher production rates than the NT treatment (Tukey, $P = 0.038$). Mean CO_2 production for the MP treatment fell between the CT and NT totals, but was not significantly different than either.

Decomposition rates did not significantly differ between tillage treatments (one-way ANOVA, $P < 0.1$; Figure 4). Comparing days 17 and 36, mean decomposition rates increased for the second half of the study for the MP, CT and S treatments and decreased for the NT treatment. However, differences were only significant for the surface-applied bags (Tukey, $P = 0.032$). Visual inspection revealed that macroinvertebrate or rodent herbivory may have caused mass loss of the surface bags (Figure 5a), whereas the incorporated litter showed more evidence of decay (Figure 5b). Also, plant roots were observed to have entered many of the buried litter bags. While care was taken to

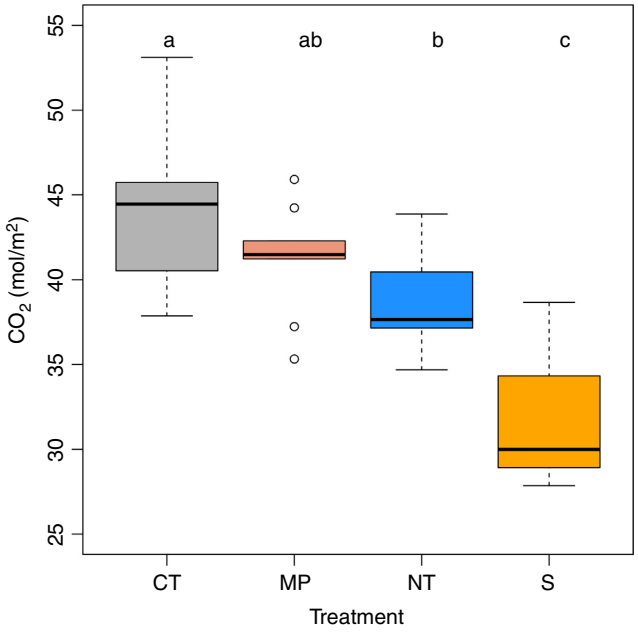

Figure 3 Mean CO_2 production (mol/m^2) from the field experiment. CT, conventional till; MP, no-till with artificial macropores; NT, no-till; S, surface-applied litter. Lower-case letters indicate statistical differences ($P < 0.1$).

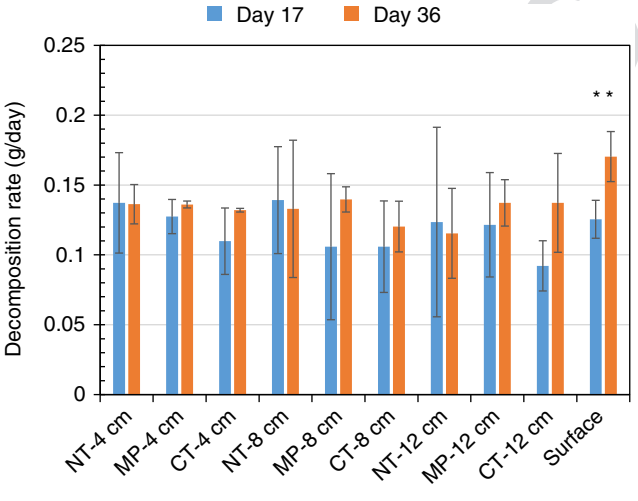

Figure 4 Litter decomposition rates (g/day) measured in the field study on days 17 (blue) and 36 (orange). CT, conventional till; MP, no-till with artificial macropores; NT, no-till; S, surface-applied litter. Depth of incorporation is indicated by the number followed by 'cm'. Error bars indicate ±1 standard deviation. *Indicates significant differences ($P < 0.1$).

remove living roots, both of these factors (predation of the above-ground residue, external biomass from roots) may have biased these results.

Column study

In the column study, the ILM treatment consistently had the highest CO_2 efflux rates (Figure 6), with statistical differences seen from one or more treatments except on days 26 and 61 (Tukey HSD, $P < 0.1$; Table 1). The incorporated litter without macropores (IL) treatment generally had the second highest fluxes, particularly when the water content was >0.30 cm^3/cm^3 (Figure 6). The ILM treatment always had higher fluxes than the IL treatment, with statistical differences ($P < 0.1$) observed for days 13–20 and 49–50. The control columns (C) always had lower respiration fluxes than either of the incorporated litter treatments, with similar magnitudes to the surface litter (SL) except when the column soil water exceeded 0.30 cm^3/cm^3 (e.g. days 11, 27 and 29). When soil water content was elevated, the surface treatment tended to have statistically higher fluxes than one or more other treatments (Table 1).

Respiration rates in the IL and ILM treatments showed variability between relatively wet and relatively dry conditions (Figure 7). For instance, CO_2 fluxes for the ILM and incorporated litter without macropores (IL) treatments were substantially reduced at high water contents (e.g. WFPS > 75%). However, specific WFPS thresholds where CO_2 efflux became curtailed (e.g. <4 μmol/m^2/s) differed between treatments, with a threshold value of ~65% for the IL treatment and ~75% for the ILM treatment. Further, the ILM treatment had its highest CO_2 effluxes when WFPS was between 55 and 70%, which was both higher and narrower than the maximum CO_2 efflux range for the IL treatment (WFPS = 40–60%). When WFPS dropped below ~50%, the two treatments provided similar respiration rates.

All four treatments showed significantly different cumulative CO_2 production values (Figure 8a; Tukey HSD, $P < 0.1$). The ILM treatment had the highest overall CO_2 production (31.3 ± 0.95 mol/m^2), followed by the IL (24.0 ± 2.1 mol/m^2), SL (20.6 ± 1.8 mol/m^2) and then C treatments (16.2 ± 1.3 mol/m^2). These production values, when multiplied by the molecular weight of carbon (12 g/mol) and the area of each column (0.031 m^2), gave mean per column carbon losses of 11.8 g (ILM), 9.04 g (IL), 7.75 g (SL) and 6.09 g (C). Relative to the control, the ILM treatment had an estimated net carbon loss of 5.69 g per column, while the IL and S treatments had respective net carbon losses of 2.94 and 1.66 g per column. Litter loss rates reflected the same pattern, with the ILM treatment showing the most litter decomposition (6.04 ± 0.43 g), followed by the IL (5.69 ± 0.49 g) and then SL (4.07 ± 1.6 g) treatments (Figure 8b). Litter loss rates statistically differed between the ILM and SL treatments (Tukey HSD, $P = 0.04$).

Discussion and conclusions

In the field study, the conventional tillage treatments had the highest respiration rates, aligning with several previous

Figure 5 Comparison of cover crop residue: (a) surface-placed litter bag, with possible predation by above-ground fauna; (b) representative samples from the surface (S) and incorporated to 8 cm depth with macropore (MP) treatments.

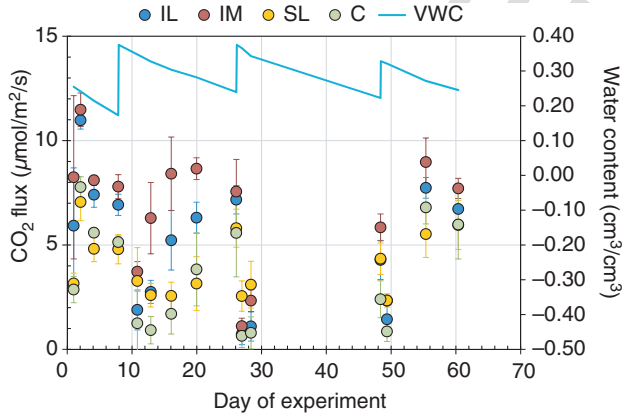

Figure 6 CO_2 flux (μmol/m^2/s) measured during the column study: IL, incorporated (8 cm depth) residue; ILM, incorporated (8 cm depth) residue with artificial macropores; SL, surface-applied litter; and C, no-litter control (n = 4). Error bars indicate ±1 standard deviation. The blue line indicates mean volumetric water content (cm^3/cm^3) from all columns.

studies (Hendrix *et al.*, 1988; Reicosky, 1997; Schwen *et al.*, 2015). The effect became more notable as the depth of incorporation increased, which could be a function of alterations in substrate availability and increases in oxygen diffusion rates that occurred throughout the profile, rather than greater decomposition of the cover crop residue. As an example, Schwen *et al.* (2015) showed that conventional tillage can enhance the effective gas diffusion rates of soil compared to no-till. Comparing no-till treatments, artificial macropores enhanced respiration rates, although the effect was only significant ($P < 0.1$) for the litter incorporated to the 12 cm depth, and for only one of eight measurements. The artificial macropores also caused higher CO_2 effluxes in the column study, with significant differences observed between the incorporated litter treatments for five sets of measurements during the 61-day study (Table 1).

The influence of macropores on soil respiration rates can be understood in the context of substrate versus oxygen diffusion limitations, such as the conceptual model provided by Skopp *et al.* (1990). In this model, substrate diffusion limits microbial activity at low water contents, oxygen diffusion constrains microbial activity at high water contents. These behaviours are reflected in Equation 4 (dashed lines in Figure 7). At low water contents, fluxes were nearly identical between the ILM and IL (i.e. with and without macropore) treatments. Substrate diffusion is controlled by water content (Nye &

Table 1 Tukey HSD *P*-values for all combinations of days and treatments in the column study

Day 1	C	S	ILM	IL	
IL	**0.092**	0.18	0.64		IL
ILM	**0.012**	**0.024**		0.72	ILM
S	0.98		**< 0.001**	**< 0.001**	S
C		0.48	**< 0.001**	**< 0.001**	C
	C	S	ILM	IL	**Day 2**

Day 26	C	S	ILM	IL	
IL	0.42	0.55	0.98		IL
ILM	0.25	0.34		0.76	ILM
S	1.0		**0.015**	**0.0028**	S
C		**0.0019**	0.65	1.0	C
	C	S	ILM	IL	**Day 27**

Day 4	C	S	ILM	IL	
IL	**< 0.001**	**< 0.001**	0.20		IL
ILM	**< 0.001**	**< 0.001**		0.16	ILM
S	0.13		**< 0.001**	**< 0.001**	S
C		0.81	**< 0.001**	**0.0026**	C
	C	S	ILM	IL	**Day 8**

Day 29	C	S	ILM	IL	
IL	0.95	**0.019**	0.19		IL
ILM	**0.078**	0.55		**0.077**	ILM
S	**0.0076**		**0.091**	1.0	S
C		**0.025**	**< 0.001**	**0.029**	C
	C	S	ILM	IL	**Day 49**

Day 11	C	S	ILM	IL	
IL	0.88	0.42	0.21		IL
ILM	**0.062**	0.95		**0.091**	ILM
S	0.14		**0.065**	1.0	S
C		**0.0088**	**< 0.001**	**0.0063**	C
	C	S	ILM	IL	**Day 13**

Day 50	C	S	ILM	IL	
IL	0.35	**0.081**	**0.086**		IL
ILM	**0.0043**	1.0		0.29	ILM
S	**0.0040**		**< 0.001**	**0.025**	S
C		0.26	**0.028**	0.51	C
	C	S	ILM	IL	**Day 56**

Day 16	C	S	ILM	IL	
IL	**0.0096**	**0.051**	**0.019**		IL
ILM	**< 0.001**	**< 0.001**		**0.064**	ILM
S	0.78		**< 0.001**	**0.019**	S
C		0.85	**< 0.001**	**0.049**	C
	C	S	ILM	IL	**Day 20**

Day 61	C	S	ILM	IL	
IL	0.35	0.34	0.99		
ILM	0.50	0.49			
S	1.0				
C					

ILM, incorporated litter with macropores. Bold indicates $P < 0.1$.

Tinker, 1977), which in dry conditions will be controlled by soil texture. Texture was the same between treatments, making it likely that substrate diffusion was also similar.

The two models diverged at high water contents (WFPS < 50%). The ILM treatment had higher observed and predicted respiration rates in these conditions, indicating that the macropores increased oxygen diffusion rates. Thus, preferential pathways, such as those created by root biopores, cracks or structure, may act to enhance soil respiration. This finding also suggests that laboratory studies performed on repacked soils might underpredict gas diffusion and soil respiration values. While macropore effects on soil gas diffusivity have been previously studied (Kristensen *et al*., 2010; Blagodatsky & Smith, 2012), these results show that macropores may need to be accounted for when modelling field-scale soil respiration and greenhouse gas emissions. Further, the artificial macropores in the columns had a total porosity of only ~0.004 cm^3/cm^3 (0.4%), making it possible that the effect seen here would strengthen as macropore porosity increases.

In general, respiration rates were significantly higher for the incorporated treatments compared to the surface-placed litter bags for both experiments. The only exception was in the laboratory experiment when soil water contents exceeded 0.30 cm^3/cm^3. It is possible that the microbes at the surface were only active when the surface soil water content was sufficiently elevated to remove substrate limitations. Those same wet conditions also reduced respiration from the bulk soil profile, likely due to reduced air-filled porosity and lower gas diffusion rates. The combination of these two processes (i.e. greater surface microbial activity and reduced soil efflux) during wet conditions may help to explain results such as those observed by MacDonald *et al*. (2010), where tilling a humid, poorly drained soil reduced respiration. Tillage in wet conditions can also decrease soil structure (Dexter & Bird, 2001), which may reduce soil CO_2 efflux

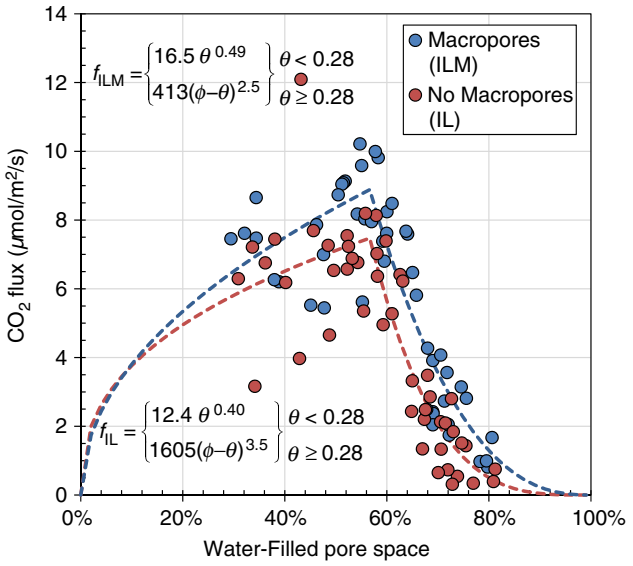

Figure 7 CO_2 flux (μmol/m^2/s) from the ILM (incorporated residue with artificial macropores) and the IL (incorporated residue with no macropores) columns versus water-filled pore space. Dashed lines represent the equations indicated by 'f_{ILM}' and 'f_{IL}'.

rates. Conversely, incorporating cover crop residue may increase respiration rates in well-drained agricultural soils.

The column study revealed larger differences in respiration rates between incorporated and surface-applied litter treatments than the field study. This result could reflect the greater level of disturbance in the column incorporation treatments (i.e. the soil was sieved and repacked and was therefore even more disturbed than the field conventional tillage treatment). The two studies also had different boundary conditions, in that the columns had a defined control volume from which respiration occurred, which was not true in the field plots. A related factor is that the field study included autotrophic respiration (from the living corn plants), which may have masked treatment differences, while the column study only included heterotrophic respiration.

The column study also revealed a possible linkage between total soil respiration and litter decomposition rates, as the ILM columns had the greatest litter loss and highest overall CO_2 production, while the surface (SL) columns had the least (Figure 8). In mass balance terms, the estimated net carbon loss from each litter treatment was within a factor of 1–2.5 times the amount of litter lost from each experiment, suggesting that the excess respiration (relative to the control) observed in the litter treatments was due to decomposition of the cover crop residue.

Altogether, the study revealed that macropores may enhance soil respiration rates in agricultural fields. Conservation agricultural practices like no tillage and cover crops have been documented to increase macropore size and connectivity (Abdollahi *et al.*, 2014), meaning that soil respiration may become enhanced as a result. On the other hand, no-till typically retains most of the crop residue near the soil surface, which may help to reduce soil respiration, particularly in well-drained soils. The elevated respiration rates resulting from macropores observed in these experiments may therefore be more muted in systems where plant litter is maintained at the

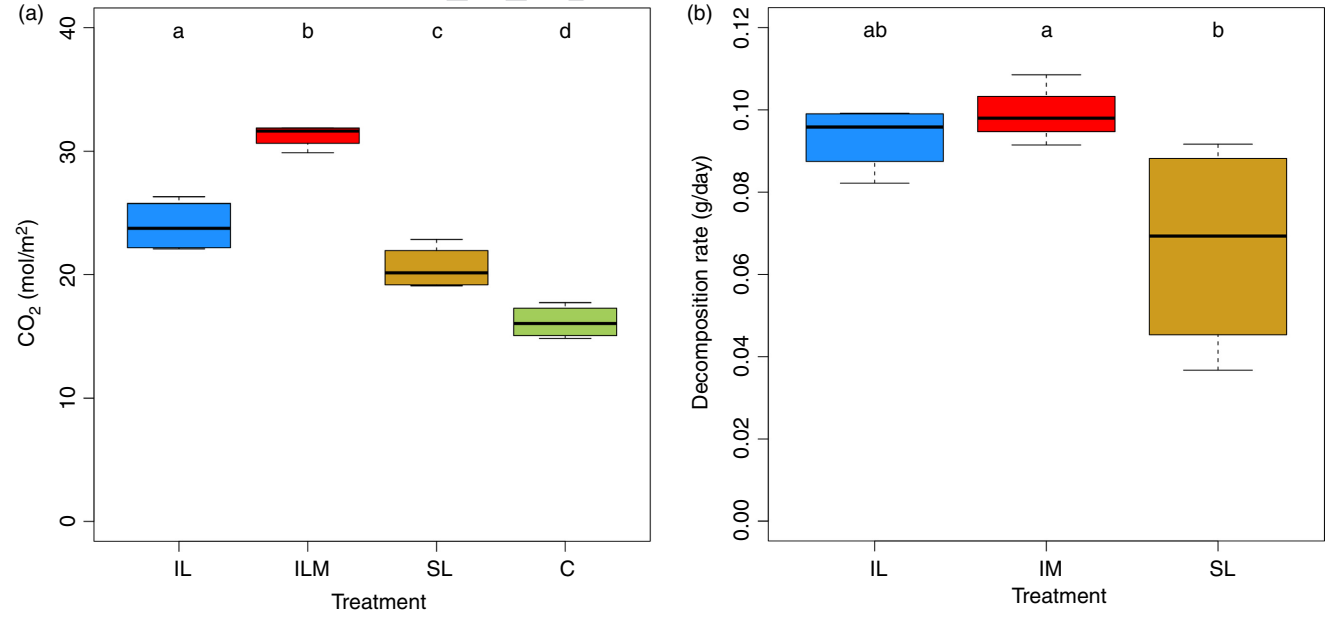

Figure 8 (a) Total CO_2 produced (mol/m^2) during the 61-day column experiment; (b) Litter loss rates (g/day) observed during the 61-day column study. Treatments: IL, incorporated litter without macropores; ILM, incorporated litter with artificial macropores; SL, surface-applied litter. Lower-case letters indicate significance ($P < 0.1$).

surface, unless macropores act as preferential pathways that allow organic compounds to migrate rapidly from the surface to depth. Such behaviours have been seen in cracking soils (Martinez *et al*., 2010), so it is possible that this process could occur in other macroporous soils.

Finally, developing soil structure is often considered to be a means of stabilizing carbon within the soil (Paustian *et al*., 2000; Govaerts *et al*., 2009), so the overall effects of macroporosity on soil carbon sequestration may vary with the type and location of carbon source, the environmental conditions and possible feedbacks such as plant roots following and reinforcing structural pores. The treatments imposed in this study all involved some disturbance, which may have altered soil organic carbon accessibility and potentially increased the apparent effects of macropores (most notably in the columns). Still, these results suggest that macropores may represent an overlooked and dynamic factor affecting soil respiration.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. CO_2 fluxes (μmol m^{-2} s^{-1}) measured during the study for litter that was incorporated at (a) 4 cm depth, (b) 8 cm depth, and (c) 12 cm depths.

Figure S2. CO_2 produced (mol m^{-2}) during the 36-day experiment for the (a) 4 cm depth; (b) 8 cm depth; and (c) 12 cm depth.