Soil fertility status controls the decomposition of litter mixture residues

JENNIFER BLESH AND TIANYU YING

School for Environment and Sustainability, University of Michigan, 440 Church Street, Ann Arbor, Michigan 48109 USA

Citation: Blesh, J., and T. Ying. 2020. Soil fertility status controls the decomposition of litter mixture residues. Ecosphere 11(8):e03237. 10.1002/ecs2.3237

Abstract. Increasing agroecosystem biodiversity with cover crops can restore many ecosystem functions that are lost with simplified crop rotations. Mixtures of species with complementary plant traits, such as legumes and grasses, may increase multiple functions at once, including soil nutrient supply and retention, which depend on microbial decomposition dynamics. Litter mixtures can stimulate decomposition compared to individual species, and decomposition also varies with soil properties. However, the interactive effect of cover crop functional type and soil fertility status on decomposition are not known. Here, we tested for mixture effects with a legume–grass litter in soils with lower and higher levels of fertility. We also identified specific soil properties that are associated with those effects. We incubated hairy vetch, cereal rye, and vetch-rye litter treatments for 360 d in two soils from fields with contrasting management histories and fertility levels, as defined by biological indicators such as particulate organic matter (POM) pools. We measured decomposition dynamics through respired CO$_2$, microbial biomass, microbial extracellular enzyme activity, and inorganic nitrogen (N) mineralization. With no litter addition, the soil with larger POM pools had twofold greater microbial biomass C, seven times more net N mineralization, and respired 58% more CO$_2$. Across both soils, after 30 d microbial biomass C increased by 58–208% following litter addition, and litter addition significantly increased CO$_2$ production compared to the no-litter control. However, there was no difference in the magnitude of CO$_2$ production among cover crop treatments and soils after litter addition. The lower fertility soil had a greater response to the litter C input for CO$_2$ production and enzyme activities in soil. Furthermore, the size and N content of free and intra-aggregate POM pools were associated with differences in the microbial response to litter addition. Our results demonstrate that cover crop litter affects microbial decomposition dynamics differently in soils with distinct soil fertility levels and suggests that new C inputs have larger effects in lower fertility soils. Understanding how soils with different fertility levels respond to diverse cover crops, including mixtures, will inform management of agroecosystems for sustainability.

Key words: cereal rye; cover crop; decomposition; extracellular enzymes; hairy vetch; nitrogen mineralization; particulate organic matter.

Received 9 May 2020; accepted 20 May 2020; final version received 18 July 2020. Corresponding Editor: Debra P. C. Peters.

Copyright: © 2020 The Authors. This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

† E-mail: jblesh@umich.edu

INTRODUCTION

Industrial agroecosystems have low plant species diversity and rely on energy-intensive inputs to sustain crop production, resulting in widespread environmental degradation (Matson 1997, Tilman et al. 2002). Increasing plant diversity in agroecosystems can improve sustainability by connecting biodiversity and ecosystem function (Isbell et al. 2017, Kremen and Merenlender 2018). Use of cover crops, or non-harvested crops grown in rotation with commercial crops, is a...
popular management practice for reintroducing plant diversity into agroecosystems (Snapp et al. 2005, Isbell et al. 2017). Distinct functional types of cover crops, such as grasses and legumes, have plant traits that may be able to restore ecosystem functions lost in highly simplified rotations. The fibrous root systems of grass species assimilate and retain soil nutrients, reducing nitrate leaching (Tonitto et al. 2006). Legume cover crops fix atmospheric nitrogen (N) through symbiotic relationships with rhizobia bacteria and provide an organic N source to agroecosystems that becomes available to subsequent crops through litter decomposition. Cover crop mixtures of legumes and grasses, which combine these complementary functional traits, can therefore reduce the need for external inputs by increasing multiple ecosystem functions simultaneously (Ranells and Wagger 1997, Finney and Kaye 2017, Blesh 2018).

Decomposition and N mineralization are fundamental processes that support ecosystem functioning in natural ecosystems (Melillo et al. 1982, Wardle et al. 1997, Hector et al. 2000). In agroecosystems, managing biogeochemical processes like decomposition to drive internal nutrient cycling can help mitigate excess additions of synthetic fertilizers (Drinkwater and Snapp 2007). Soil decomposition dynamics are shaped by interactions between climate and soil conditions, the size and composition of the microbial community, and litter quality (i.e., physical and chemical properties) and quantity (Parton et al. 2007, Zhang et al. 2008, Wickings et al. 2012, Allison et al. 2013). The effects of cover crop litter inputs on soil nutrient cycling dynamics are therefore mediated by microbial decomposition processes. Legumes have N-rich litter that releases N quickly upon incorporation into the soil (Melillo et al. 1982, Hector et al. 2000, White et al. 2017). Yet, rapid decomposition of legume biomass in agroecosystems can lead to N losses if N mineralization is not synchronized with growth of the subsequent crop (Crews and Peoples 2005, Basche et al. 2014). Combining legumes with grass cover crops has the potential to slow net N mineralization rates and improve synchrony of nutrient cycling, because the microbial community immobilizes soil N to meet its physiological requirements when decomposing grass litter with a high C:N ratio.

Litter mixtures, however, can also stimulate microbial decomposition and overall N release through non-additive effects (Wardle et al. 1997, Wardle and Barret 1999, Hector et al. 2000). Although effects vary widely, studies on decomposition of litter mixtures in natural ecosystems have often shown a positive effect of mixed residues on decomposition compared to the individual species (Wardle et al. 1997, Barret and Shine 1999, Gartner and Cardon 2004). Potential mechanisms for these mixture effects include greater physical and chemical complexity of litter mixtures, which may increase the diversity of microbial habitats and niche partitioning, or transfer of nutrients between plant species via fungal hyphae (Frey et al. 2000, Gartner and Cardon 2004, Hättenschwiler et al. 2005). One study on the mixture effect in an agroecosystem found positive effects of crop residue mixtures on CO2 production (McDaniel et al. 2016). However, most litter mixture studies have been conducted with perennial species spanning a relatively wide range of substrate qualities. While annual legume and grass cover crops have contrasting functional traits, they are grown for a relatively short duration in managed ecosystems, which also have a greater frequency of disturbance than many natural ecosystems. There is a need, then, to understand how litter mixture inputs affect decomposition in the context of cover cropping, a management practice with the potential to increase nutrient availability to harvested crops.

The decomposition of cover crop litter is also expected to vary with soil characteristics, particularly soil fertility (Stark et al. 2008, Bowles et al. 2014). In agroecosystems, soil fertility can be defined by indicators that reflect biological processes such as turnover of organic matter and nutrient cycling (Marriott and Wander 2006a, Hrusso et al. 2016). For instance, two soils that have similar properties, such as C:N or texture, may have different levels of biological activity as a result of unique management legacies. Over time, diversified management is expected to increase soil organic matter (SOM) stocks, particularly labile C pools such as particulate organic matter (POM). POM fractions are important for internal nutrient cycling and soil organic C storage because they provide an energy source for microbial decomposition (Wander et al. 1994, Marriott and Wander 2006b).
agroecosystems often have improved N retention in soil (Gardner and Drinkwater 2009) that co-occurs with high levels of N cycling between organic matter, soil inorganic N pools, and plants (Drinkwater and Snapp 2007), which can be quantified with other biological indicators of fertility such as potentially mineralizable N (Drinkwater et al. 1996). Changes in microbial communities and faster cycling SOM fractions with diversified management likely have feedbacks on the decomposition of new C inputs to soil. However, to date, the interactive effects of cover crop residue mixtures and soil fertility levels on decomposition dynamics have not been tested. One incubation study testing the litter mixture effect in agroecosystems showed that a history of previous crop diversity had a greater mixture effect in agroecosystems showed that a history of previous crop diversity had a greater effect on CO2 production than did recent crop residue inputs to soil (McDaniel et al. 2016). Furthermore, this legacy of increased diversity increased decomposition due to changes in soil fertility (McDaniel et al. 2014). However, to our knowledge, no studies have identified specific soil fertility measures that influence how new litter inputs affect soil decomposition dynamics. This is critical information for determining how cover crops will impact soil biogeochemical processes in different contexts, and therefore for quantifying potential benefits of an important conservation practice.

Here, we addressed this knowledge gap by measuring decomposition dynamics of legume and grass cover crop litter incubated for 360 d in two soils from fields with different long-term management histories and distinct fertility levels, as characterized by total organic C, nutrient cycling capacity, and POM pools. This design allowed us to determine how the short-term effects of transitions to cover cropping interact with background soil fertility and to identify soil properties that are associated with these effects. We focused on a legume cover crop, hairy vetch (*Vicia villosa* L.), and a grass, cereal rye (*Secale cereale* L.), because they are complementary plant functional types, and two of the most commonly grown cover crops in temperate agroecosystems. Specifically, we tested the following three hypotheses: (1) The mixed vetch-rye litter would have the greatest net N mineralization, microbial enzyme activity, and CO2 production due to the mixture effect; (2) the microbial response (i.e., CO2 production, enzyme activity) to litter C inputs would be greater in the lower fertility soil relative to the higher fertility soil; and (3) the soil with larger POM pools would have greater overall microbial CO2 production and N release, both with and without litter addition.

**MATERIALS AND METHODS**

**Field histories**

To test our hypotheses, we designed an aerobic incubation experiment using soil and litter collected from two farm fields in southeastern Michigan with contrasting levels of soil fertility, likely due to their different long-term management histories (Appendix S1: Table S1). Both fields had the same short-term (i.e., two-year) history, because they were involved in a companion experiment with the same organic management regime, which included two winters with a hairy vetch–cereal rye cover crop mixture when we began the current study (Blesh 2019). However, the fields had different long-term legacies. The higher fertility field had been in certified organic garlic production for six years before the on-farm experiment, with a regular sequence of garlic and six non-harvested cover crop species in a three-year rotation. Prior to that, the field had been fallow for 25 yr. The lower fertility field, in contrast, had been in organic mixed vegetable production for three years, with no cover crop use before establishing the on-farm experiment. Prior to that, it had been in continuous alfalfa hay production for over a decade (Appendix S1: Table S1).

The experimental fields on the two farms were planted in a mixture of cereal rye (seeding rate of 56 kg/ha) and hairy vetch (seeding rate of 25 kg/ha) on 2 September and 14 September 2016. Seeds were surface broadcast and lightly incorporated, and each field was divided into four replicate blocks.

**Soil analyses and baseline soil fertility**

The fields were selected based on significant differences in multiple biological indicators of soil fertility (Table 1). A baseline, composite soil sample from each block in each farm field was collected to 20 cm depth at the end of the vetch-rye cover crop season (on 16 May and 18 May 2017, immediately before incorporation). A
Table 1. Initial properties of the two soils in the experiment.

| Property                          | Higher     | Lower     |
|-----------------------------------|------------|-----------|
| OM (%)                            | 3.7 (0.2)  | 1.6 (0.1) |
| Free POM (g/kg)                   | 12.1 (1.3) | 3.9 (0.9) |
| Free POM C (mg/kg)               | 190.8 (22.5) | 45.3 (6.4) |
| Protected POM (g/kg)             | 8.8 (0.7)  | 4.5 (0.9) |
| Protected POM C (mg/kg)          | 149.3 (6.5) | 71.6 (6.1) |
| PMN (mg/kg, week^{-1})           | 13.1 (1.6) | 5.9 (1.0) |
| NH_{4}^{+} + NO_{3}^{-} (mg/kg)  | 1.5 (0.2)  | 0.8 (0.1) |
| P (mg/kg)                        | 36.5 (6.5) | 33.5 (2.1) |
| K (mg/kg)                        | 102.0 (17.5) | 78.3 (7.0) |
| pH                               | 7.8 (0.1)  | 7.0 (0.1) |
| CEC (meq/100 g)                  | 10.5 (0.8) | 5.6 (0.2) |
| Sand (%)                         | 60.5 (1.3) | 74.0 (1.8) |
| Silt (%)                         | 23.5 (1.0) | 15.0 (1.3) |
| Clay (%)                         | 16.0 (0.8) | 11.0 (0.6) |
| Soil series                      | Miami B    | Fox sandy Loam A |

Notes: Values are means ± standard error. Abbreviations: CEC, cation exchange capacity; OM, organic matter; PMN, potentially mineralizable nitrogen; POM, particulate organic matter.

subset of ~100 g of sieved, dried soil was analyzed for particle size (texture), percentage of organic matter, pH, Bray-1 P, K, and other macro- and micro-nutrients at the A & L Great Lakes Laboratories (Fort Wayne, Indiana, USA). The soil from the field with a history of a long fallow followed by organic management and regular cover crop use had higher total SOM compared to the farm that had a more intensive long-term production history (3.7% vs. 1.6%; Table 1). We also analyzed fractions of SOM that are important for microbial decomposition and nutrient cycling, and which tend to reflect different management histories (Drinkwater et al. 1996, Ward 2004). Light fraction particulate organic matter (POM; also called free POM), and intra-aggregate POM (i.e., physically protected, or occluded POM), was separated from triplicate 40 g subsamples of air-dried soil using a size and density fractionation method (Marriott and Ward 2006b, Blesk 2019). We retained material larger than 53 µm, and the C and N of free POM and protected POM were measured on an ECS 4010 CHNSO Analyzer (Costech Analytical Technologies; Valencia, California, USA). Free POM in the higher fertility soil (12.1 g/kg) was three times higher than in the lower fertility soil (3.9 g/kg). Moreover, the N concentration of the free POM pool was four times higher in the higher fertility soil, and the physically protected POM N concentration was twofold greater in the higher fertility soil (Table 1). The size of the physically protected POM pool was also slightly larger on the higher fertility soil (P = 0.062). Triplicate soil subsamples were sieved for inorganic N analysis (see Soil-litter incubation below), and for an anaerobic N mineralization incubation—potentially mineralizable N (PMN), which is an indicator of N availability from decomposition. Approximately 8 g fresh soil was incubated anaerobically in 10 mL of deionized water for 7 d followed by extraction with 2 mol/L KCl (Drinkwater et al. 1996). PMN was calculated by subtracting the initial amount of NH_{4}^{+} in the soil from the NH_{4}^{+} released during the 7-d incubation. The rate of potentially mineralizable N (PMN) was more than twofold greater in the higher fertility soil (13.1 vs. 5.9 mg N·kg^{-1}·week^{-1}). In terms of soil properties that reflect soil type rather than management history, percentage of sand was higher in the lower fertility soil (74 vs. 61), and percentage of clay was higher in the more fertile soil (16 vs. 11). The soil series on the higher fertility field was Miami, a fine-loamy, mixed, active, mesic Oxyaquic Hapludalf. The soils are superactive, mesic Typic Hapludalf. The soils are both Alfisols; however, the Fox series has outwash soils that are better drained than the Miami series.

Soil-litter incubation

Soil for use in the incubation was collected on 31 April 2017 from the experimental field in the vetch-rye mixture on both farms approximately two weeks before cover crop termination by tillage. A composite sample of eight to ten, 10 cm soil cores (2 cm diameter) were homogenized from each of the four replicate blocks per farm field, and half of the soil was sieved to 2 mm. To measure the initial soil inorganic N pool, approximately 8 g of sieved soil was added to triplicate, 50-mL centrifuge tubes containing 40 mL of 2 mol/L KCl for extraction of nitrate (NO_{3}^{-}) and ammonium (NH_{4}^{+}). The tubes and remaining soil were stored in a cooler on ice until they were brought back to the laboratory. A subsample of soil was set aside for analysis of
baseline microbial biomass and microbial enzyme activities and was refrigerated for up to 72 h. The remaining fresh soils were sieved to 2 mm and divided into 8 mason jars (4 replicate blocks × 2 farms). After measuring soil moisture, soils were brought to 50% water holding capacity and pre-incubated for five days without any plant residue to minimize the effects of disturbance due to our sampling before starting the decomposition incubation (McDaniel et al. 2014).

For the incubation, we used dried and ground vetch and rye litter from the two fields, which had been collected from monoculture plots of each species in the first year of the experiment in May 2016. Each replicate block per field had included three microplots of 1 m² with sole vetch, sole rye, and vetch-rye mix treatments (Blesh 2019). The biomass was sampled to ground level from a 0.25 m² quadrat in each plot in each field, avoiding soil, immediately before cover crop incorporation. The litter was dried at 60°C for three days, ground to 2 mm in a Wiley mill, and stored until use in the incubation. Initial litter C and N were determined by dry combustion on a Leco TruMac CN Analyzer (Leco, St. Joseph, Michigan, USA). Initial concentrations of lignin, cellulose, and hemicellulose for each litter treatment were measured using acid detergent fiber (ADF) and neutral detergent fiber (NDF) methods at the University of Wisconsin, Madison, Soil and Forage Analysis Laboratory (Marshfield, Wisconsin, USA).

To establish the incubation mesocosms, 50-mL centrifuge tubes were labeled and their weights were recorded. We took 500 g of pre-incubated soil (400 g dry soil equivalent) from each block on each farm and divided it equally among four, 1-L mason jars. To the mason jars, except for the control, we added 1.2 g of the dry plant litter for each treatment (rye, vetch, or vetch-rye mixture), to simulate a high litter input rate in an agroecosystem. For the mixture treatment, we combined 0.54 g vetch with 0.66 g rye litter, which matched the proportion of vetch and rye biomass in the mixture growing in the field. After soils were well-mixed with litter, each soil-residue treatment was then divided evenly into three 50-mL tubes (for three destructive sampling dates), and the weight of each tube was recorded. The three tubes for each treatment, and the control with no plant residue, were placed in a Mason jar and incubated in a dark room at approximately 23°C for 360 d. We used the tube weights to track and maintain the soil at 50% water holding capacity using DI water.

**Soil inorganic N pools**

We also extracted soil inorganic N (NH₄⁺-N + NO₃⁻-N) on day 30 of the incubation period with 2 mol/L KCl. On day 30, one tube was destructively sampled from each mason jar. We measured out 16 g of soil from each tube and evenly divided it into two replicate test tubes. We added 40 mL of 2 mol/L KCl to each tube. Tubes were placed on a shaker for one hour, centrifuged for 10 min, and 20 mL of supernatant was collected with a pipette and frozen in vials until analysis. The amount of NH₄⁺ and NO₃⁻ in each sample was analyzed colorimetrically on a continuous flow analyzer (AQ2; Seal Analytical, Mequon, Wisconsin, USA). Net N mineralization over the first 30 d was calculated as soil inorganic N (NH₄+ + NO₃⁻) on day 30 minus initial soil inorganic N. Remaining soil from the sampled tubes was immediately processed for microbial biomass and frozen for subsequent extracellular enzyme analysis.

**Microbial biomass C and N**

Like soil inorganic N pools, we measured microbial biomass C and N on days 0 and 30 only, following previous studies observing a steep decline in microbial activity after 30 d (McDaniel et al. 2014). Microbial biomass was estimated using the chloroform-fumigation extraction method (Vance et al. 1987) as modified by Fierer and Schimel (2003). On day 0, we measured out 10 g soil into two replicate tubes. We added 40 mL 0.5 mol/L K₂SO₄ to both replicates, and 0.5 mL chloroform to one of the replicates and capped the tubes immediately in a fume hood. On day 30, we repeated this procedure, but with 5 g of soil and 20 mL 0.5 mol/L K₂SO₄ in each tube. All tubes were placed on a reciprocal shaker at 180 oscillations per min for 4 h and then centrifuged at 252 g for 10 min. The solution was filtered using Whatman No. 1 filter papers into 20 mL vials, frozen at −20°C. Residual chloroform was removed by air-bubbling for 15 min before analysis on a Shimadzu TOC-TN (Shimadzu Scientific Instruments, Columbia, Maryland, USA).
CO₂ production measurements

Soil respiration rates were measured by the amount of CO₂ produced in a given period, for a total of 20 time points over the incubation. At each time point, we first uncapped the lids of all mason jars and let them sit for a half hour to equilibrate with ambient CO₂ in the laboratory, and we recorded the initial CO₂ concentration on a LI-820 CO₂ Gas Analyzer (LI-COR Biosciences, Lincoln, Nebraska, USA). The mason jars were then recapped using lids fitted with rubber septa and incubated at 23°C in the dark. Over the first two weeks, the CO₂ production measurement time was approximately 3 h. After the respiration rate decreased, the CO₂ production measurement time increased to 6 h and eventually to 48 h. Accordingly, the interval between tests increased from once per day, to once per month after four months. A syringe was used to extract gas from each mason jar through a rubber septum, which was immediately injected into the LI-820 to measure CO₂ concentration. The CO₂ production data were processed by taking the difference in CO₂ concentration between time 1 (start) and time 2 (end). The CO₂ concentration at each time point was first corrected and converted to mass unit (Cm) using the ideal gas law (Holland et al. 1999). The CO₂ production (P) was then calculated with the change in CO₂-C mass over the incubation period (Cm), the headspace volume (L), the mass of DI water added to tubes (Ww) to maintain the soil at 50% water holding capacity, the mass of soil in the mason jar before adding water (m), and the actual incubation time (t):

\[ P = \frac{C_m \times (L - \frac{W_w}{1050})}{m \times t}. \]

The CO₂ production was adjusted with the standard reading (1.01% CO₂) to get the actual CO₂ production for each sample (µg CO₂-C/g/day). The cumulative CO₂ production during the whole incubation was then calculated using with the total number of time points (N), the CO₂ production at two adjacent time points (f_{d-1} and f_d), and the number of days between these two time points (g):

\[ CO_2 = \sum_{k=1}^{n} \frac{f_{d-1} + f_d}{2} \times g. \]

Data analysis

Calculation of carbon production.—The raw CO₂ production data were processed by taking the difference in CO₂ concentration between time 1 (start) and time 2 (end). The CO₂ concentration at each time point was first corrected and converted to mass unit (Cm) using the ideal gas law (Holland et al. 1999). The CO₂ production (P) was then calculated with the change in CO₂-C mass over the incubation period (Cm), the headspace volume (L), the mass of DI water added to tubes (Ww) to maintain the soil at 50% water holding capacity, the mass of soil in the mason jar before adding water (m), and the actual incubation time (t):

\[ P = \frac{C_m \times (L - \frac{W_w}{1050})}{m \times t}. \]

The CO₂ production was adjusted with the standard reading (1.01% CO₂) to get the actual CO₂ production for each sample (µg CO₂-C/g/day). The cumulative CO₂ production during the whole incubation was then calculated using with the total number of time points (N), the CO₂ production at two adjacent time points (f_{d-1} and f_d), and the number of days between these two time points (g):

\[ CO_2 = \sum_{k=1}^{n} \frac{f_{d-1} + f_d}{2} \times g. \]

Calculation of mixture effects.—We calculated the mixture effect for the rye-vetch litter for each response variable according to the equation...
(\(O - E\))/E (Meier and Bowman 2010, McDaniel et al. 2016), where \(O\) is the observed response for each replicate (\(n = 4\)) within the mixture treatment and \(E\) is the expected response. \(E\) was calculated for each response variable by taking the mean value of the sole vetch and sole rye treatments for each replicate.

**Statistical analysis.**—Statistical analysis was conducted in R (R Core Team 2017) and SAS Software, Version 9.4 (SAS Institute 2017). We determined the relative importance of farm soil (higher and lower fertility) and litter treatment (control, mix, rye, vetch) on all response variables at each sampling date with linear models using the lm function in R. The response variables tested were soil inorganic N and microbial biomass C, N, and C:N on days 0 and 30; extracellular enzyme activity of each enzyme (BG, NAG, PHOS, PHENOX) for days 0, 30, and 90; and CO\(_2\) production by each incubation date sampled (e.g., day 2, day 25, day 360). Specifically, we used a two-way ANOVA, blocked by field block, with farm and treatment as fixed effects, along with their interaction. The assumptions of normality, independence, and equal variance were met. Comparison of least square means was performed using Tukey’s honestly significant difference (HSD). We ran an additional one-way ANOVA to test for differences in response variables between the two soils on day 0, before litter treatments were imposed, with field and block as fixed effects. We tested for non-additive effects of the rye-vetch litter mixture on microbial biomass C, N, and C:N, soil inorganic N pools, and extracellular enzyme activities after 30 d, and on cumulative CO\(_2\) production on days 25 and day 360, with one-way \(t\)-tests to determine if \((O - E)/E\) values were significantly different from zero. We also used one-way ANOVA models to compare mixture effects for the two soil treatments. Finally, to test whether soil fertility properties on the two fields (i.e., POM pools and potentially mineralizable N) influenced the effect of litter addition on CO\(_2\) production, we first calculated a mean effect size of litter addition that combined the three litter treatments. The effect size was calculated using the cumulative (360 d) CO\(_2\) production data as ln(treatment) – ln(control) by field and block. We then used a one-way ANOVA model in R to determine differences in the mean effect size, with field and block as fixed factors. We used the same one-way ANOVA model to test for differences in the measured soil fertility indicators across the two farms. Results are reported as statistically significant at \(\alpha = 0.05\).

**RESULTS**

**Litter chemistry**

The plant litter for the incubation came from the 2016 season of the on-farm experiment. In that year, the cover crops were incorporated relatively early in the spring (on April 27 and April 28). Analysis of litter chemical traits indicated that rye litter had higher hemicellulose, and lower lignin and cellulose concentrations compared to the vetch litter; however, the rye and vetch litter had similar C:N ratios (Table 2). The vetch-rye mixture had intermediate values of cellulose and hemicellulose, and the same C:N ratio as the sole rye litter (Table 2).

**Inorganic N and microbial biomass**

Concentrations of NH\(_4^+\) were relatively low at both measured time points (e.g., day 30: mean NH\(_4^+\)-N was 0.99 ± 0.1 and 2.36 ± 0.1 mg N/kg dry soil on higher and lower fertility soils, respectively), so we summed soil NO\(_3^-\) and NH\(_4^+\) concentrations as the total extractable inorganic

### Table 2. Initial litter chemistry for the three cover crop residue treatments in the incubation (means of the two farm fields).

| Treatment          | C:N   | C   | N   | Lignin | Cellulose | Hemicellulose |
|--------------------|-------|-----|-----|--------|-----------|---------------|
| Rye                | 22.38 | 42.13 | 1.92 | 1.79   | 23.22     | 22.76         |
| Vetch              | 16.07 | 36.11 | 2.37 | 3.83   | 41.15     | 4.17          |
| Mixture (vetch-rye)| 21.69 | 39.44 | 1.96 | 2.81   | 27.53     | 16.3          |

**Notes:** Litter was collected from sole-planted plots of each species in the first year of an associated field experiment in May 2016. The mixture treatment was composed of 45% vetch and 55% rye litter. Values for C, N, lignin, cellulose, and hemicellulose are percentages.
N pool. Before litter addition (day 0), the higher fertility soil had higher inorganic N compared to the lower fertility soil ($P = 0.039$), although overall inorganic N concentrations were low (Fig. 1A). The amount of net N mineralized over 30 d in each soil was therefore similar to the extractable inorganic N pool size on day 30. At day 30, soil inorganic N in the no-litter control was much higher for the higher fertility soil ($50.6 \pm 5.0$ vs. $7.4 \pm 0.9$ mg/kg dry soil ($P < 0.0001$). The higher fertility control had $7 \times$ greater net N mineralization than the lower fertility control. Litter addition, however, did not lead to a significant increase in inorganic N compared to the control for any of the litter treatments in either soil. Averaging across treatments, including the control, the higher fertility soil had $9 \times$ the inorganic N (in mg/kg dry soil) compared to the lower fertility soil.

Microbial biomass carbon (MBC) was not different between the two soils on day 0 ($P = 0.425$), but was significantly greater on the higher fertility soil on day 30 ($P < 0.0001$; Fig. 1B). At day 30, the higher fertility control revealed a strong incubation effect on MBC, whereas MBC in the lower fertility control did not change from day 0. MBC generally increased over the first 30 d of the incubation with cover crop litter addition compared to the controls (Fig. 1B). On the higher fertility soil, the three plant litter treatments had 58–104% higher MBC compared to the no-litter control, and on the lower fertility soil, litter treatments had an even larger effect, with 100–204% higher MBC than the control. Microbial biomass N (MBN) followed a similar pattern, with no difference between soils at day 0, but a highly significant difference between the two soils ($P = 0.0003$) by day 30, with twofold more MBN in the higher fertility soil (mean of $10.4 \pm 1.1$ vs. $4.9 \pm 0.6$ mg/kg soil). However, there were no differences in MBN among litter treatments on day 30. The ratios of MBC to MBN (MBC:MBN) were not different on day 0 ($10.4 \pm 1.9$ vs. $9.6 \pm 1.7$ for lower and higher fertility soils, respectively). On day 30, across all treatments, the lower fertility soil had a significantly greater mean MBC:MBN ($12.8 \pm 1.3$) compared to the higher fertility soil ($9.1 \pm 0.6$; $P = 0.0002$). The higher fertility soil had no difference in MBC:MBN between treatments and the control, but, for the lower fertility soil, the MBC:MBN was significantly lower in the mixture and vetch treatments compared to the control, and the ratio was also significantly lower for the mixture compared to rye (mix: $8.3 \pm 1.5$, vetch: $11.3 \pm 2.2$, rye: $14.7 \pm 1.0$, control: $19.9 \pm 0.8$; $P = 0.019$).

**Carbon dynamics**

Without litter addition, the lower and higher fertility soils had significant differences in CO$_2$ production over the course of the incubation...
The no-litter control on the higher fertility soil had 57.9% greater production of CO$_2$ compared to the lower fertility control. For cover crop litter treatments, cumulative CO$_2$ production ranged from 5196 to 6524 µg CO$_2$-C g$^{-1}$ dry per soil over the 360-d incubation. Overall, the CO$_2$ production was much higher with the added plant litter than without, indicating a significant response in microbial activity to the fresh C input ($P < 0.0001$; Fig. 2). However, the cumulative CO$_2$ production did not differ significantly among the three cover crop residue treatments. Following litter addition, CO$_2$ production from the two soils also was not different ($P = 0.421$).

The first three to four weeks of the incubation were the optimal period for microbial enzyme production, plant residue decomposition, and CO$_2$ respiration, so we expected most of the differences in respiration between soil and litter treatments to occur during this time. On day 25, the difference in cumulative CO$_2$ production between the no-litter control and the litter treatments was highly significant for both soils ($P < 0.0001$); however, there were no significant differences among the three litter treatments, or between the two soils. Cumulative CO$_2$ respiration for the first 25 d of the incubation ranged from 3067 to 3774 µg CO$_2$-C/g dry soil for all soils.

![Cumulative CO$_2$ production](image.png)

**Fig. 2.** Cumulative CO$_2$ production for all treatments on the two soils over the 360-d incubation (A) and on days 25 and 360 (B). Low = lower fertility soil; high = higher fertility soil. An asterisk indicates a significant difference between soils.
treatments with added litter, compared to a range of 210–519 µg CO₂-C/g dry soil with no litter. The difference between the no-litter controls in the higher fertility and lower fertility soils was nearly statistically significant ($P = 0.06$; Fig. 2b). After 25 d, the respiration rate slowed down.

**Soil fertility properties and CO₂ production**

To gain a more mechanistic understanding of the effect of litter addition, we tested whether differences in the baseline soil fertility measures influenced the CO₂ response to litter addition in the two soils. Given no observed differences in CO₂ production among litter treatments, we combined the three treatments for analysis. Overall, the mean effect size of CO₂ response to cover crop residue addition (litter addition – control) was larger than 0 (Fig. 3) and significantly greater on the lower fertility soil ($P = 0.005$). Four of the soil fertility parameters measured at the baseline sampling, which reflect nutrient cycling capacity, were significantly different across the two soils—free POM ($P = 0.021$), the free POM N pool ($P = 0.009$), the protected POM N pool ($P = 0.002$), and the rate of potentially mineralizable N (PMN; $P = 0.006$)—and showed that lower soil fertility was associated with a larger effect size (Fig. 3).

![Graphs showing the mean effect of cover crop residue treatments compared to the control on cumulative CO₂ production and the mean of four different biological measures of soil fertility.](image)

Fig. 3. The mean effect of the cover crop residue treatments ($t$, litter addition) compared to the control ($c$, no litter addition) on cumulative CO₂ production (± SD) vs. the mean of four different biological measures of soil fertility (± SD) on the two soils in the incubation. Low = lower fertility soil; high = higher fertility soil. POM = particulate organic matter; PMN = potentially mineralizable N.
**Enzyme dynamics**

For each enzyme, we calculated the ratio of enzyme activity to microbial biomass C (MBC) because of inherent differences in soil fertility across the two soils. Per unit MBC, the higher fertility soil had higher initial enzyme activities for the three hydrolytic enzymes (NAG, BG, PHOS) compared to the lower fertility soil (Fig. 4), but this difference was only statistically significant for PHOS ($P = 0.049$). Conversely, for PHENOX enzyme activity, which reflects the potential decay of recalcitrant C compounds (e.g., lignin), the lower fertility soil had a significantly higher enzyme activity per unit MBC on day 0 compared to the higher fertility soil ($P = 0.009$; Fig. 4). By day 30, however, activities for the three hydrolytic enzymes were significantly greater relative to MBC in the lower fertility soil than in the higher fertility soil (Fig. 4), which was driven by the smaller MBC pool in the lower fertility soil (Fig. 1B). On day 30, pairwise comparisons showed that enzyme activities for the plant litter treatments were not significantly different from each other, or from the control, in the higher fertility soil. In the lower fertility soil, there were also almost no differences among the three plant treatments, but EEA:MBC ratios were significantly greater in the no-litter control compared to the litter treatments (Fig. 4), whereas absolute enzyme activities tended to be higher with litter addition compared to the control in this soil (Appendix S1: Fig. S1). On the lower fertility soil, PHENOX activity per MBC was approximately fivefold lower on day 30 for all treatments compared to initial activity on day 0.

![Figure 4](image-url)

**Fig. 4.** Mean extracellular enzyme activity (EEA):microbial biomass C (MBC) ratios of the higher and lower fertility soils before cover crop litter addition (day 0) and after 30 d of the incubation (day 30) by soil and litter treatment ($\pm$ SE). Low = lower fertility soil; high = higher fertility soil. The enzyme abbreviations are NAG = $\beta$-1,4-N-acetyl glucosaminidase; BG = $\beta$-1,4-glucosidase; PHOS = acid phosphatase; and PHENOX = phenol oxidase. Different lowercase letters indicate significant differences between litter treatments within a field at $P < 0.05$. The table inset shows $P$ values testing for differences between the two soils on day 0 and day 30.
day 0. In contrast, PHENOX activity in the higher fertility soil did not change after 30 d in any of the treatments.

We also compared ratios of BG: NAG and BG: PHENOX activities over 90 d to assess patterns of C and nutrient acquisition in the two different soils (Fig. 5). For this analysis, we combined litter treatments because there were no differences in absolute enzyme activity across them on day 30 or 90 (Appendix S1: Fig. S1). For the higher fertility soil, the ratio of BG: NAG—an indicator of microbial demand for C vs. N—did not change between day 0 and day 30. After day 30 the ratio decreased, with BG and NAG activity becoming similar at day 90. At day 30 in the lower fertility soil, the relative NAG activity was higher than at day 0 (BG:NAG decreased) and then did not change between day 30 and day 90. The difference in this ratio between the two soils was only significant on day 90 ($P < 0.0001$).

For the BG: PHENOX ratio (i.e., labile C to recalcitrant C-degrading enzymes), the microbial community in the lower fertility soil produced relatively more BG over time. In the higher fertility soil, the BG: PHENOX ratio declined after day 30. The difference in BG:PHENOX between the higher and lower fertility soils was significant on day 0 ($P = 0.022$) and on day 90 of the incubation ($P < 0.0001$), but not on day 30.

**Mixture effects**

On average, the vetch-rye litter mixture did not result in non-additive mixture effects for any response variable (Appendix S1: Table S2). None of the mixture effect indices were significantly different from 0, nor were there any significant differences in the indices across the two soils (Appendix S1: Table S2). The effect of the litter mixture on cumulative respiration was especially small and ranged from 12% less respiration than expected to 8% more respiration than expected for the mixture on day 25, compared to the average of the rye and vetch litters decomposing alone. On day 360, this range was even smaller: from 8% lower to 4% higher cumulative CO$_2$ production than expected for the mixture. In other cases, large variation in the direction and magnitude of the mixture effect indices across replicates resulted in no significant mean mixture effects for measured responses. For instance, there was a slight suppression of PHENOX production in the mixture, on average, compared to the sole litter treatments; however, it was not statistically significant. Similarly, the mixture tended to increase MBN and decrease the MBC:MBN ratio, with a larger effect in the lower fertility soil, which also had higher than expected extractable inorganic N concentrations, on average. However, these effects were not significantly

![Fig. 5](image_url)

Fig. 5. Mean BG:NAG (left) and BG:PHENOX (right) ratios during the first 90 d of the incubation for the higher (high) and lower fertility (low) soils (± SE). The enzyme abbreviations are NAG = β-1,4-N-acetyl glucosaminidase; BG = β-1,4-glucosidase; and PHENOX = phenol oxidase.
different for the two soils, or different from 0, due to large variation across replicates.

**DISCUSSION**

We directly tested the effects of cover crop functional diversity and soil fertility status on microbial responses to litter addition through a laboratory mesocosm experiment. We quantified a set of synergistic outcomes—CO₂ production, changes in inorganic N pools and microbial biomass, and extracellular enzyme activity—to capture different components of litter decomposition. We aimed to understand interactions between the short-term effects of transitioning to cover crop mixtures and background soil conditions, by decomposing cover crop residues in two soils with different long-term management histories, and associated differences in soil fertility status. Although residue addition had a strong effect on CO₂ production, our hypothesis about positive mixture effects with legume–grass litter addition was not supported. However, similar to previous studies comparing conventional and organic farm management, we found that soil fertility status affected microbial responses to new C inputs, with cascading impacts on soil biogeochemical processes (Gunapala et al. 1998, Berthrong et al. 2013, Bowles et al. 2014). It is now well established that biological measures of soil fertility, such as POM pools, are responsive to changes in agroecosystem management (Wander 2004, Marriott and Wander 2006a). Here, for the first time, our results show the important role of POM in altering microbial responses to soil incubation and new litter C inputs. Specifically, we found support for our hypothesis that the higher fertility soil would have greater CO₂ production and N release in the absence of litter inputs, due to the POM C and nutrients made available to microbes under incubation conditions. However, with cover crop litter addition there were no differences in cumulative CO₂ production between the two soils, even though the higher fertility soil had larger MBC, MBN, and extractable inorganic N pools. Furthermore, on day 30 of the incubation, extracellular enzyme activity-to-MBC ratios were also greater on the lower fertility soil. These findings support our hypothesis that microbial responses to C inputs from cover crop litter would be greater on a lower fertility soil. Understanding this context, dependency is crucial for tailoring agroecosystem management practices such as cover cropping to different environmental conditions across farms.

**Effects of functionally diverse cover crop mixtures**

A common finding in litter decomposition studies, particularly in natural ecosystems, is a mixture effect, where mixed litter residues stimulate decomposition rates (Wardle et al. 1997, Bardgett and Shine 1999, Gartner and Cardon 2004). Litter mixtures have more chemical complexity than individual residues, which may affect how they are decomposed (Meier and Bowman 2008). As distinct functional types, cereal rye and hairy vetch residues typically have contrasting biochemical traits (Ranells and Waggener 1996, Poffenbarger et al. 2015). We therefore predicted that vetch-rye litter would produce the greatest CO₂ and release the most N during the course of the incubation because of the mixture effect. These responses would indicate greater microbial activity, which can drive longer-term accumulation of soil organic C through multiple mechanisms (Schmidt et al. 2011, Cotrufo et al. 2013).

While we found that litter addition increased the size of the microbial biomass pool and CO₂ production compared to the no-litter control, in contrast to our hypothesis, we found almost no support for positive mixture effects, especially for cumulative CO₂ production. Other studies have found limited effects of litter mixtures on microbial respiration, but larger effects on soil N cycling, particularly N mineralization and the MBN pool (Meier and Bowman 2010). Similarly, the mean mixture effect indices for our study (Appendix S1: Table S2) suggest a narrower MBC:MBN ratio and a larger extractable inorganic N pool with the cover crop mixture, particularly in the lower fertility soil; however, the mixture effects were highly variable across replicates within a treatment and were not significantly different from zero. Similarly, results from our ANOVA models showed that the lower fertility soil had the lowest MBC:MBN ratio in the mixture treatment, which was significantly smaller than the no-litter control and the sole rye, but was not different from sole vetch.
It is possible that we found no mixture effects on decomposition dynamics because the vetch and rye litter had similar C:N ratios in our experiment. On the two farms, the cover crop mixture was incorporated relatively early in the spring because of the timing of planting the following crop. As a result, rye plants were in a vegetative growth stage at sampling, and the rye and vetch litter did not have the contrasts in C:N ratio, and N (%) that we expected based on their plant functional types, despite some differences in chemical traits between the two cover crop species (Table 2). Therefore, the effects of cover crop litter on soil processes also depend on management practices; for instance, the timing of residue incorporation impacts litter chemical traits and the actual functional diversity of mixtures. Other mixture decomposition studies have used litter with a wide range of biochemical composition (Wardle et al. 1997, McDaniel et al. 2016). We would thus expect treatment differences with larger contrasts in litter chemistry (Meier and Bowman 2008), even though cover crops are typically high-quality substrates. However, it is also possible that the artificial conditions of a laboratory incubation experiment would mask treatment differences even for cover crops with greater biochemical contrasts. The litter was dried, ground, and mixed into soil to create homogeneous conditions, which eliminates the physical heterogeneity typical of litter mixtures. We also excluded root material, which would increase the biochemical complexity of litter inputs. In field conditions with living plants, rhizosphere interactions can stimulate decomposition and N mineralization (Cheng 2009, Kuzyakov 2010). Litter treatment differences, and potentially mixture effects, are therefore likely to be stronger in field conditions. For instance, other studies at the field scale have shown that microbial activity increases with greater aboveground diversity in agroecosystems (Gunapala et al. 1998, Pimentel et al. 2005, Tiemann et al. 2015).

Effects of soil fertility status

Previous studies have found that the effects of litter mixtures on decomposition depend on other factors besides litter chemistry, particularly soil properties (e.g., Jonsson and Wardle 2008). In this study, soil fertility had a large effect on decomposition dynamics. The baseline measures of POM fractions and potentially mineralizable N contrasted strongly on the two farm fields (Table 1, Fig. 3), likely due to their distinct management legacies (Appendix S1: Table S1). Free, or light fraction, POM is derived from recent litter or other C inputs to soil (e.g., manure or compost) and turns over on annual timescales, while intra-aggregate POM is physically protected from microbial decomposition and turns over on decadal time scales (Wander et al. 1994, von Lützow et al. 2006, Marrriott and Wander 2006). However, following sieving (i.e., breaking up aggregates) and incubating the soils, C and nutrients from both POM pools would likely be more available to the microbial community compared to field conditions.

Our results highlight the important role of POM as a driver of divergent responses to the soil-litter incubation in the two soils. For instance, both soils experienced an incubation effect on net N mineralization between day 0 and day 30 (i.e., an increase in soil inorganic N, but no difference between the control and treatments with litter inputs); however, in the higher fertility soil, the incubation effect was much greater, with nine times more soil inorganic N, on average, than the lower fertility soil (Fig. 1A). We expected greater N mineralization in the higher fertility soil given its larger initial pools of potentially mineralizable N and POM N (Table 1). A previous incubation study found that legume litter stimulated N mineralization regardless of management history (Stark et al. 2008). However, we found relatively small increases in soil inorganic N on the lower fertility soil compared to the baseline measurement (day 0), suggesting that the microbial community was more N limited in the incubation with the lower fertility soil. While soil fertility status had a large effect on inorganic N, litter functional type had little to no effect in either soil, likely due to the similar chemical properties of the vetch and rye residues, the fact that the litter was ground and mixed with soil, or both. In field conditions, on the other hand, prior studies have shown that vetch cover crop litter increased short-term soil N availability more than vetch-rye mixtures and rye monocultures (Kuo and Sainju 1998, Poffenbarger et al. 2015).

In contrast to patterns in extractable inorganic N, litter addition did increase MBC in both soils compared to the control, indicating an increase
in the microbial biomass pool. Microbial biomass is controlled by C availability (Zak et al. 1994, Kallenbach and Grandy 2011), and cover crop litter addition provides a high-quality C source for microbial decomposition and associated soil nutrient cycling processes. There was a strong incubation effect on MBC in the higher fertility soil, and overall, the MBC pool was approximately twofold larger than in the lower fertility soil, reflecting the larger pools of available C and nutrients in that soil. On the other hand, the lower fertility soil exhibited no change in MBC in the control between days 0 and 30, but had a larger percent increase in MBC with new C inputs from litter. Even small increases in microbial biomass drive critical ecosystem functions. For instance, microbial biomass can increase N availability in the rhizosphere through cycles of assimilation and mineralization, and via turnover of the microbial biomass (Manzoni and Porporato 2009). The importance of microbial byproducts and necromass for soil organic C storage is also increasingly recognized (von Lützow et al. 2006, Cotrufo et al. 2013). The different responses across the two soils potentially signal a more C-limited microbial community in the lower fertility soil, which was also supported by its higher PHENOX activity at baseline (Fig. 4). The significantly larger MBC:MBN ratios on day 30 suggest that the lower fertility soil was also more N limited than the higher fertility soil during the incubation, which corresponds with the large difference in soil inorganic N between the two soils.

Different categories of extracellular enzymes, such as hydrolytic and oxidative, degrade different organic C compounds. Therefore, changes in extracellular enzyme activities (EEA) in soil reflect the types of compounds that the microbial community is decomposing to acquire energy and nutrients, and can also signal nutrient limitation in the soil (Sinsabaugh et al. 2005). Over the first 30 d of the incubation, changes in EEA, expressed per unit MBC, supported the role of soil fertility in driving divergent responses across the soils. There was a greater microbial response to litter C addition in terms of EEA on the lower fertility soil, which corresponds with the results for CO₂ production. On day 0, the two soils had the same size MBC pool, but initial EEA followed expected patterns based on the soil fertility differences. Previous studies have found higher activities for hydrolytic enzymes that break down labile organic compounds in agroecosystems with a history of organic management (Mader et al. 2002, Moeskops et al. 2010), which matches our findings for the higher fertility soil at baseline (Fig. 4; Appendix S1: Fig. S1). The higher initial PHENOX activity in the lower fertility soil, in contrast, indicates a microbial physiology adapted to accessing more recalcitrant C sources (Sinsabaugh 2010). On day 30, however, the opposite pattern emerged with the lower fertility soil showing higher EEA:MBC ratios for the hydrolytic enzymes, suggesting the microbial community was investing more C in enzyme production than the higher fertility soil, especially in the no-litter control. This strong incubation effect on EEA:MBC for the lower fertility soil was driven by the small MBC pool in the lower fertility control and suggests microbes were allocating even more C to enzyme production without litter C addition. The response to both incubation conditions and litter C inputs was also evidenced by the sharp decline in the PHENOX: MBC ratio on the lower fertility soil after 30 d, which tended to be even lower for the litter treatments than for the control. In contrast, the lack of differences in EEA between control and litter treatments on the higher fertility soil, even though the size of the MBC pool increased, suggests that the litter input mattered little relative to C and nutrients accessible from other organic matter pools and that the microbial community in this soil invested less energy in enzyme production.

To better understand the status of soil nutrient availability given the different fertility levels, we also analyzed ratios of enzyme activities for the two soils (McDaniel et al. 2014). On the higher fertility soil, the change in the ratio of BG:NAG over time suggests an increase in microbial demand for N after 30 d (Fig. 5). By contrast, in the lower fertility soil N limitation appeared to occur earlier, with a decline in BG:NAG by day 30, which corresponded with an increase in the mean MBC:MBN ratio from day 0. These divergent patterns make sense given the larger initial pools of C and N, such as POM, in the higher fertility soil (Table 1), as well as the much higher concentrations of extractable inorganic N in that soil on day 30. The contrasting trajectories for the
ratio of hydrolytic to oxidative enzymes (BG:PHENOX) over the first 90 d (Fig. 5) also point to a greater response to litter C input on the lower fertility soil, which started with smaller POM pools and higher PHENOX activity. On the higher fertility soil, the decrease in this ratio after 30 d suggests a shift toward reliance on more recalcitrant substrates after more accessible fractions of C were decomposed. However, on the lower fertility soil, the increase in the BG:PHENOX ratio over 90 d suggests that the microbial community continued accessing more labile C following incubation conditions and litter C inputs.

In addition to EEA, soil fertility also had a large effect on microbial respiration, as measured by CO₂ production over time. Without litter addition, the higher fertility soil produced more cumulative CO₂ over 360 d than the lower fertility soil, which followed our hypothesis based on their different background levels of POM. This shows the strong effect of the size and quality of SOM pools on decomposition (Bowles et al. 2014). Also as predicted, following litter addition we observed a greater response to the fresh C input in the lower fertility soil (Figs. 2, 3) where microbes may have been more C and nutrient limited. However, we expected greater CO₂ production from the higher fertility soil overall, and this was only the case without litter inputs. Instead, with litter addition, respiration on the lower fertility soil matched that in the higher fertility soil, despite having a smaller microbial biomass pool at day 30. Assessing microbial physiology was outside of the scope of this study, although these results suggest potential differences in microbial C allocation (i.e., for respiration and enzyme production vs. growth and reproduction). A previous experiment comparing agroecosystems with distinct management legacies found approximately 50% higher microbial C use efficiency and microbial growth rates in an organic system with a long-term history of diverse plant inputs, which also had greater SOC accumulation (Kallenbach et al. 2015).

Our analysis of the combined effect sizes comparing litter treatments and controls also showed the greater response to litter C inputs—with greater CO₂ production—on the lower fertility soil. A previous incubation study found that soil with a history of low crop diversity had the greatest increase in cumulative CO₂ production following the addition of a crop litter mixture (McDaniel et al. 2016). Other incubation studies have found that microbial communities on lower fertility soils have a strong response to new C inputs, stimulating their biomass and activity regardless of management history (Gunapala et al. 1998, Stark et al. 2008). Our study identified specific indicators of soil fertility that were associated with this difference in effect size (Fig. 3): more rapidly cycling fractions of SOM (i.e., POM), POM N concentration, and potentially mineralizable N rates. The different effect sizes across the two farms indicate that there may be particular thresholds of key soil fertility indicators at which the microbial response to fresh C addition increases rapidly, which is an important area for future research. With respect to management practices, our findings suggest that farmers with lower fertility soils could realize key short-term benefits to soil biological processes following cover crop adoption, even though increasing the total SOM pool can take 10 yr or longer (Drinkwater et al. 1998, Syswerda et al. 2011).

Soil fertility indicators, such as POM and related labile C and N pools, respond relatively quickly to changes in management and are considered strong indicators of both soil nutrient cycling and C stabilization (Marriott and Wander 2006a, Hurisso et al. 2016, Blesh 2019). Accumulation of these organic matter pools, which include the microbial biomass itself, affect and are affected by the microbial community. The specific mechanisms driving these relationships between management practices and agroecosystem function are not fully understood, but most likely involve changes in microbial community composition (i.e., linkages between above- and below-ground biodiversity; Schlatter et al. 2015), changes in resources available to support microbial activity (Bowles et al. 2014), or changes in microbial physiological traits (Kallenbach et al. 2015). The relationships we found between soil fertility and decomposition inform the management of legume–grass cover crops to enhance N mineralization from organic matter and ultimately reduce the use of synthetic inputs such as N fertilizer. Improving predictive understanding of decomposition dynamics, by assessing these relationships across wider gradients of environmental conditions, could ultimately improve the
management of aboveground diversity for ecosystem functions in agriculture.

CONCLUSIONS

Soil decomposition dynamics have cascading impacts on the stabilization of C and N in SOM, reduction of N losses through nitrate leaching and N₂O emissions, and associated sustainability of agroecosystems. In this study, we did not find evidence for synergistic mixture effects on decomposition with a functionally diverse cover crop, likely due to small differences in C:N ratios between the legume and grass litter. Future studies should test cover crop residues with greater contrasts in C:N ratio to better understand how cover crop functional trait diversity impacts decomposition. However, by measuring several complementary components of soil decomposition we found that soil fertility status alters the breakdown of newly added cover crop litter, due to differences in the size and N content of SOM pools, especially POM pools with year to decadal turnover times. The greater total N release and MBC following incubation in the higher fertility soil highlight the benefits of long-term cover crop use for enhancing soil nutrient cycling and potentially sequestering atmospheric CO₂. On the other hand, we found a greater microbial response to new C inputs in soil with lower fertility status, highlighting the importance of conservation practices for restoring ecosystem functions in degraded soils. Our results demonstrate the importance of understanding how organic nutrient sources, such as legume cover crops, impact decomposition, and associated ecological outcomes in different soil conditions, which can inform targeting conservation practices to where they will be most effective.

ACKNOWLEDGMENTS

Support for this research was provided by the Ceres Trust Organic Research Initiative and by the School for Environment and Sustainability at the University of Michigan. We thank the farmer collaborators in this research, and Beth VanDusen, Brendan O’Neill, and Eliot Jackson for assistance in the field and laboratory, and Inés Ibáñez, Brendan O’Neill, and two anonymous reviewers for suggestions that improved the manuscript.

LITERATURE CITED

Allison, S. D., Y. Lu, C. Weihe, M. L. Goulden, A. C. Martiny, K. K. Treseder, and J. B. Martiny. 2013. Microbial abundance and composition influence litter decomposition response to environmental change. Ecology 94:714–725.

Bardgett, R. D., and A. Shine. 1999. Linkages between plant litter diversity, soil microbial biomass and ecosystem function in temperate grasslands. Soil Biology and Biochemistry 31:317–321.

Basche, A. D., F. E. Miguez, T. C. Kaspar, and M. J. Castellano. 2014. Do cover crops increase or decrease nitrous oxide emissions? A meta-analysis. Journal of Soil and Water Conservation 69:471–482.

Berthrong, S. T., D. H. Buckley, and L. E. Drinkwater. 2013. Agricultural management and labile carbon additions affect soil microbial community structure and interact with carbon and nitrogen cycling. Microbial Ecology 66:158–170.

Blesh, J. 2018. Functional traits in cover crop mixtures: biological nitrogen fixation and multifunctionality. Journal of Applied Ecology 55:38–48.

Blesh, J. 2019. Feedbacks between nitrogen fixation and soil organic matter increase ecosystem functions in diversified agroecosystems. Ecological Applications. https://doi.org/10.1002/eap.1986

Bowles, T. M., V. Acosta-Martinez, F. Calderón, and L. E. Jackson. 2014. Soil enzyme activities, microbial communities, and carbon and nitrogen availability in organic agroecosystems across an intensively-managed agricultural landscape. Soil Biology and Biochemistry 68:252–262.

Cheng, W. 2009. Rhizosphere priming effect: its functional relationships with microbial turnover, evapotranspiration, and C-N budgets. Soil Biology and Biochemistry 41:1795–1801.

Cotrufo, M. F., M. D. Wallenstein, C. M. Boot, K. Denef, and E. Paul. 2013. The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: Do labile plant inputs form stable soil organic matter? Global Change Biology 19:988–995.

Crews, T. E., and M. B. Peoples. 2005. Can the synchrony of nitrogen supply and crop demand be improved in legume and fertilizer-based agroecosystems? A review. Nutrient Cycling in Agroecosystems 72:101–120.

Drinkwater, L. E., C. A. Cambardella, J. D. Reeder, and C. W. Rice. 1996. Potentially mineralizable nitrogen as an indicator of biologically active soil nitrogen. Pages 217–229 in J. W. Doran and A. J. Jones, editors. Methods for assessing soil quality. SSA Special Publication 49, Madison, Wisconsin, USA.
Drinkwater, L. E., and S. S. Snapp. 2007. Nutrients in agroecosystems: rethinking the management paradigm. Advances in Agronomy 92:163–186.
Drinkwater, L. E., P. Wagoner, and M. Sarrantonio. 1998. Legume-based cropping systems have reduced carbon and nitrogen losses. Nature 396:262–265.
Fierer, N., and J. P. Schimel. 2003. A proposed mechanism for the pulse in carbon dioxide production commonly observed following the rapid rewetting of a dry soil. Soil Science Society of America Journal 67:798–805.
Finney, D. M., and J. P. Kaye. 2017. Functional diversity in cover crop polycultures increases multifunctionality of an agricultural system. Journal of Applied Ecology 54:509–517.
Frey, S., E. Elliott, K. Paustian, and G. Peterson. 2000. Fungal translocation as a mechanism for soil nitrogen inputs to surface residue decomposition in a no-tillage agroecosystem. Soil Biology and Biochemistry 32:689–698.
Gardner, J. B., and L. E. Drinkwater. 2009. The fate of nitrogen in grain cropping systems: a meta-analysis of 15N field experiments. Ecological Applications 19:2167–2184.
Gartner, T. B., and Z. G. Cardon. 2004. Decomposition dynamics in mixed-species leaf litter. Oikos 104:230–246.
Gardner, J. B., and L. E. Drinkwater. 2009. The fate of nitrogen in grain cropping systems: a meta-analysis of 15N field experiments. Ecological Applications 19:2167–2184.
Gartner, T. B., and Z. G. Cardon. 2004. Decomposition dynamics in mixed-species leaf litter. Oikos 104:230–246.
German, D. P., M. N. Weintraub, A. S. Grandy, C. L. Lauber, Z. L. Rinkes, and S. D. Allison. 2011. Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. Soil Biology and Biochemistry 43:1387–1397.
Gunapala, N., R. Venette, H. Ferris, and K. Scow. 1998. Effects of soil management history on the rate of organic matter decomposition. Soil Biology and Biochemistry 30:1917–1927.
Hättenschwiler, S., A. V. Tiunov, and S. Scheu. 2005. Biodiversity and litter decomposition in terrestrial ecosystems. Annual Review of Ecology, Evolution, and Systematics 36:191–218.
Hector, A., A. Beale, A. Minns, S. Otway, and J. Lawton. 2000. Consequences of the reduction of plant diversity for litter decomposition: effects through litter quality and microenvironment. Oikos 90:357–371.
Holland, E. A., G. P. Robertson, J. Greenberg, P. M. Groffman, R. D. Boone, and J. R. Gosz. 1999. Soil CO2, N2O, and CH4 exchange. Pages 185–201 in G. P. Robertson, D. C. Coleman, C. S. Bledsoe, and P. Sollins, editors. Standard soil methods for long-term ecological research. Oxford University Press, New York, New York, USA.
Hursso, T. T., S. W. Culman, W. R. Horwath, J. Wade, D. Cass, J. W. Beniston, T. M. Bowles, A. S. Grandy, A. J. Franzluebbers, and M. E. Schipanski. 2016. Comparison of permanganate-oxidizable carbon and mineralizable carbon for assessment of organic matter stabilization and mineralization. Soil Science Society of America Journal 80:1352–1364.
Isbell, F., P. R. Adler, N. Eisenhauer, D. Fornara, K. Kimmel, C. Kremen, D. K. Letourneau, M. Liebman, H. W. Polley, and S. Quijas. 2017. Benefits of increasing plant diversity in sustainable agroecosystems. Journal of Ecology 105:871–879.
Jonsson, M., and D. A. Wardle. 2008. Context dependency of litter-mixing effects on decomposition and nutrient release across a long-term chronosequence. Oikos 117:1674–1682.
Kallenbach, C., and A. S. Grandy. 2011. Controls over soil microbial biomass responses to carbon amendments in agricultural systems: a meta-analysis. Agriculture, Ecosystems & Environment 144:241–252.
Kallenbach, C., A. Grandy, S. Frey, and A. Diefendorf. 2015. Microbial physiology and necromass regulate agricultural soil carbon accumulation. Soil Biology and Biochemistry 91:279–290.
Kremen, C., and A. Merenlender. 2018. Landscapes that work for biodiversity and people. Science 362:eaau6020.
Kuo, S., and U. Sainju. 1998. Nitrogen mineralization and availability of mixed leguminous and non-leguminous cover crop residues in soil. Biology and Fertility of Soils 26:346–353.
Kuzyakov, Y. 2010. Priming effects: interactions between living and dead organic matter. Soil Biology and Biochemistry 42:1363–1371.
Lee, Y. B., N. Lorenz, L. K. Dick, and R. P. Dick. 2007. Cold storage and pretreatment incubation effects on soil microbial properties. Soil Science Society of America Journal 71:1299–1305.
Mader, P., A. Fliessbach, D. Dubois, L. Gunst, P. Fried, and U. Niggli. 2002. Soil fertility and biodiversity in organic farming. Science 296:1694–1697.
Manzoni, S., and A. Porporato. 2009. Soil carbon and nitrogen mineralization: theory and models across scales. Soil Biology and Biochemistry 41:1355–1379.
Marriott, E. E., and M. Wander. 2006a. Qualitative and quantitative differences in particulate organic matter fractions in organic and conventional farming systems. Soil Biology and Biochemistry 38:1527–1536.
Marriott, E. E., and M. M. Wander. 2006b. Total and labile soil organic matter in organic and conventional farming systems. Soil Science Society of America Journal 70:950–959.
Matson, P. A. 1997. Agricultural intensification and ecosystem properties. Science 277:504–509.
McDaniel, M., A. Grandy, L. Tiemann, and M. Weintraub. 2014. Crop rotation complexity regulates the decomposition of high and low quality residues. Soil Biology and Biochemistry 78:243–254.

McDaniel, M., A. Grandy, L. Tiemann, and M. Weintraub. 2016. Eleven years of crop diversification alters decomposition dynamics of litter mixtures incubated with soil. Ecosphere 7:e01426.

Meier, C. L., and W. D. Bowman. 2008. Links between plant litter chemistry, species diversity, and belowground ecosystem function. Proceedings of the National Academy of Sciences of the United States of America 105:19780–19785.

Meier, C. L., and W. D. Bowman. 2010. Chemical composition and diversity influence non-additive effects of litter mixtures on soil carbon and nitrogen cycling: implications for plant species loss. Soil Biology and Biochemistry 42:1447–1454.

Melillo, J. M., J. D. Aber, and J. F. Muratore. 1982. Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. Ecology 63:621–626.

Moeskops, B., D. Buchan, S. Sleutel, L. Herawaty, E. Husen, R. Saraswati, D. Setyorini, and S. De Neve. 2010. Soil microbial communities and activities under intensive organic and conventional vegetable farming in West Java, Indonesia. Applied Soil Ecology 45:112–120.

Parton, W., W. L. Silver, I. C. Burke, L. Grassens, M. E. Harmon, W. S. Currie, J. Y. King, E. C. Adair, L. A. Brandt, and S. C. Hart. 2007. Global-scale similarities in nitrogen release patterns during long-term decomposition. Science 315:361–364.

Pimentel, D., P. Hepperly, J. Hanson, D. Douds, and R. Seidel. 2005. Environmental, energetic, and economic comparisons of organic and conventional farming systems. BioScience 55:573–582.

Poffenbarger, H. J., S. B. Mirsky, R. R. Weil, J. E. Maul, M. Kramer, J. T. Spargo, and M. A. Cavigelli. 2015. Biomass and nitrogen content of hairy vetch–cereal rye cover crop mixtures as influenced by species proportions. Agronomy Journal 107:2069–2082.

R Core Team. 2017. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/

Ranells, N. N., and M. G. Waggar. 1996. Nitrogen release from grass and legume cover crop monocultures and bicultures. Agronomy Journal 88:777–882.

Ranells, N. N., and M. G. Waggar. 1997. Grass-legume bicultures as winter annual cover crops. Agronomy Journal 89:659–665.

Saïva-Cork, K., R. Sinsabaugh, and D. Zak. 2002. The effects of long term nitrogen deposition on extracellular enzyme activity in an Acer saccharum forest soil. Soil Biology and Biochemistry 34:1309–1315.

SAS Institute. 2017. The SAS system for Windows. Release 9.4. Cary, NC, USA: SAS Institute Inc.

Schlatter, D. C., M. G. Bakker, J. M. Bradeen, and L. L. Kinkel. 2015. Plant community richness and microbial interactions structure bacterial communities in soil. Ecology 96:134–142.

Schmidt, M. W., et al. 2011. Persistence of soil organic matter as an ecosystem property. Nature 478:49–56.

Sinsabaugh, R. L. 2010. Phenol oxidase, peroxidase, and organic matter dynamics of soil. Soil Biology and Biochemistry 42:391–404.

Sinsabaugh, R. L., M. E. Gallo, C. Lauber, M. P. Waldrop, and D. R. Zak. 2005. Extracellular enzyme activities and soil organic matter dynamics for northern hardwood forests receiving simulated nitrogen deposition. Biogeochemistry 75:201–215.

Snapp, S. S., S. M. Swinton, R. Labarta, D. Mutch, J. R. Black, R. Leep, J. Nyiraneza, and K. O’Neill. 2005. Evaluating cover crops for benefits, costs and performance within cropping system niches. Agronomy Journal 97:322–332.

Stark, C. H., L. M. Condron, M. O’Callaghan, A. Stewart, and H. J. Di. 2008. Differences in soil enzyme activities, microbial community structure and short-term nitrogen mineralisation resulting from farm management history and organic matter amendments. Soil Biology and Biochemistry 40:1352–1363.

Syswerda, S., A. Corbin, D. Mokma, A. Kravchenko, and G. Robertson. 2011. Agricultural management and soil carbon storage in surface vs. deep layers. Soil Science Society of America Journal 75:92–101.

Tiemann, L., A. Grandy, E. Atkinson, E. Marin-Spiotta, and M. McDaniel. 2015. Crop rotational diversity enhances belowground communities and functions in an agroecosystem. Ecology Letters 18:761–771.

Tilman, D., K. G. Cassman, P. A. Matson, R. Naylor, and S. Polasky. 2002. Agricultural sustainability and intensive production practices. Nature 418:671–677.

Tonitto, C., M. B. David, and L. E. Drinkwater. 2006. Replacing bare fallows with cover crops in fertilizer-intensive cropping systems: a meta-analysis of crop yield and N dynamics. Agriculture, Ecosystems and Environment 112:58–72.

Vance, E., P. Brookes, and D. Jenkinson. 1987. An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry 19:703–707.
von Lützow, M., I. Kögel-Knabner, K. Ekschmitt, E. Matzner, G. Guggenberger, B. Marschner, and H. Flessa. 2006. Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions—a review. European Journal of Soil Science 57:426–445.

Wander, M. 2004. Soil organic matter fractions and their relevance to soil function. Soil organic matter in sustainable agriculture. CRC Press, Boca Raton, Florida, USA.

Wander, M., S. Traina, B. Stinner, and S. Peters. 1994. Organic and conventional management effects on biologically active soil organic matter pools. Soil Science Society of America Journal 58:1130–1139.

Wardle, D., K. Bonner, and K. Nicholson. 1997. Biodiversity and plant litter: experimental evidence which does not support the view that enhanced species richness improves ecosystem function. Oikos 79:247–258.

White, C. M., S. T. DuPont, M. Hautau, D. Hartman, D. M. Finney, B. Bradley, J. C. LaChance, and J. P. Kaye. 2017. Managing the trade off between nitrogen supply and retention with cover crop mixtures. Agriculture, Ecosystems & Environment 237:121–133.

Wickings, K., A. S. Grandy, S. C. Reed, and C. C. Cleveland. 2012. The origin of litter chemical complexity during decomposition. Ecology Letters 15:1180–1188.

Zak, D. R., D. Tilman, R. R. Parmenter, C. W. Rice, F. M. Fisher, J. Vose, D. Milchunas, and C. W. Martin. 1994. Plant production and soil microorganisms in late-successional ecosystems: a continental-scale study. Ecology 75:2333–2347.

Zhang, D., D. Hui, Y. Luo, and G. Zhou. 2008. Rates of litter decomposition in terrestrial ecosystems: global patterns and controlling factors. Journal of Plant Ecology 1:85–93.

**Supporting Information**

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.3237/full