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Short-Term Effects of Cover Crops on Soil Microbial Characteristics and Biogeochemical Processes across Actively Managed Farms

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Core Ideas

- The effect of cover crops on soil microbes and biogeochemistry was examined.
- Cover crops increase microbial biomass and bioavailable soil carbon.
- Increasing cover crop biomass amplifies belowground effects.

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ABSTRACT

Agricultural soils are largely degraded or under threat of degradation. Given a growing human population and the subsequent need to feed this population, agricultural practices must maintain productivity and soil quality. Cover cropping regimes are a management approach that aims to address these dual goals. Although the use of cover crops has been linked to many positive effects on soil quality and crop yields, few studies have examined their effects on soil microbial community structure and function under active farm management. We assessed soil characteristics and microbial community structure and function between agricultural field plots with and without cover crops. We expected microbes would respond in the short-term to increasing cover crop biomass, with increases in microbial activity and a shift in C acquisition toward substrates indicative of root exudation. In the presence of cover crops, we found active microbial biomass and bioavailable-C increased by 64 and 37%, respectively, indicating the potential for increased C sequestration. Soil NH_4^+ increased by 64%, whereas soil NO_3^- decreased by 30%, indicating a shift toward less mobile N forms and the potential of greater nutrient retention under cover cropping regimes. Additionally, increasing cover crop biomass was related to lower microbial biomass C/N ratios and to decreased utilization of recalcitrant C substrates. These results potentially suggest a shift toward greater microbial utilization of root-derived compounds with increasing cover crop biomass. Together, these results indicate that, in the short-term, the presence of cover crops may improve soil quality, as measured by indices of microbial activity, and soil C and nutrients.

Abbreviations: CFE, chloroform fumigation extraction; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; SIR, substrate-induced respiration; SOM, soil organic matter; WHC, water holding capacity.

Croplands make up ~20% of total land area in the United States, representing a significant source of revenue and food production (Bigelow and Borchers, 2017). However, the soil associated with these croplands is often either degraded or under threat of degradation (Lal, 1997, 2002). In fact, intensive agriculture is associated with increased rates of soil erosion (USDA–NRCS, 2010), increased nutrient runoff (Tilman et al., 2002), and decreased diversity of soil faunal communities (Tsiafouli et al., 2015). With a growing human population demanding even more agricultural intensification, efforts must be used that will lead to adequate food production while staving off soil degradation. To accomplish this, many agricultural management techniques (e.g., no-till management) have been suggested (Moebius-Clune et al., 2016). The use of cover crops (i.e., the planting of non-cash crops during the fallow period) is one such technique that has recently grown in popularity (CTIC, 2017).

Cover cropping is expected to lead to more sustainable agricultural practices by mitigating the negative effects of agricultural practices on soils (CTIC, 2017; Dabney et al., 2001). Specifically, cover crop regimes have been found to reduce soil erosion and increase nutrient retention (Dabney et al., 2001; Reicosky and Forcella, 1998). Inclusion of cover crops in cropping systems during fallow periods has the potential to sequester ~0.13 Pg C yr^{-1} globally through fresh plant inputs to the soil (Poeplau and Don, 2015). Yet, the implementation of cover cropping regimes is not without its limitations. Specifically, cover crops may need to acquire a specific level of biomass to markedly effect soil C (Ruis and Blanco-Canqui, 2017) and retain sufficient N in biomass (Dean and Weil, 2009). Achieving such biomass can be constrained by the grower's ability to plant cover crops soon after harvesting the primary crop

and allowing the cover crop sufficient time to grow before termination (Ruis and Blanco-Canqui, 2017). Additionally, the timeframe of cover crop growth is likely to be mediated by myriad factors, especially on active farms, where weather constraints and management goals can impede the full expression of cover crop effects on soil health and subsequent crop production.

Incomplete knowledge about cover cropping effects on soil microbial communities is a significant gap because soil microbial communities are key drivers of soil function (Dabney et al., 2001; Fierer, 2017; Nivelle et al., 2016; Reicosky and Forcella, 1998). For instance, the soil microbial community has been linked to decomposition (Strickland et al., 2009) and the formation of soil organic matter (SOM) (Bradford et al., 2013; Kallenbach et al., 2016). Additionally, soil microbial communities directly influence plant productivity by mineralizing soil N and mobilizing soil P (Pereg and McMillan, 2015; Vessey, 2003). In fact, cover cropping tends to increase microbial biomass, extracellular enzyme activity, and glomalin, an indicator of arbuscular mycorrhizal fungi (Balota et al., 2014). These findings are promising, but improved understanding of the short-term effects cover crops have on microbial community structure and function is still needed. For instance, cover cropping may increase belowground plant inputs, such as low-molecular-weight C compounds found in root exudates, and these inputs are expected to result in greater microbial efficiency and growth (Bais et al., 2006; Strickland et al., 2015b; van der Putten et al., 2013). However, whether soil microbial community structure and function respond rapidly to these potential cover crop effects is still relatively unknown.

Here we examine cover cropping regimes across multiple active working farms to assess whether the effects of cover crops on soil characteristics, microbial community composition, and function can be detected in a short timeframe (i.e., rapid identification of factors indicative of cover crop management). Specifically, we compared plots with and without cover crops and determined fungal-to-bacterial dominance, active microbial biomass, microbial biomass C and N, and microbial community function via catabolic profiling. We also determined an array of soil characteristics, including soil N pools and mineralizable soil C (an indicator of the bioavailable soil C pool). Furthermore, we examined the relationship between soil and microbial characteristics and cover crop biomass. Objectives for this study were to determine (i) how cover crops influence soil microbial community structure and function and key soil characteristics and (ii) if and how cover crop biomass is related to microbial and soil factors. We expected that cover crops would lead to a more active and efficient microbial community that derives the bulk of its C from root exudates. For soil characteristics, we expected greater bioavailable C and evidence of greater N retention under cover crop regimes. Finally, we expected that these effects would be amplified by increasing cover crop biomass. That is, if cover crops improve soil microbial structure and function, then we expected increasing cover crop biomass to be positively correlated with even greater improvements.

MATERIALS AND METHODS

Site Description and Experimental Design

To examine the effects of cover crops on soil microbial community structure and function and soil characteristics, we compared plots with and without cover crops on actively managed (i.e., grower-owned) agricultural fields across four farms located in Virginia (Supplemental Fig. S1; Table 1). Farms 1, 3, and 4 were in the Ridge and Valley ecoregion of Virginia; Farm 2 was in the Piedmont ecoregion (Table 1). Plots on Farms 1, 2, and 3 were located on Ultisol soils; plots on Farm 4 were located on Alfisol soils (Table 1). The texture class for all soils was silt loam, and the primary crop for all four farms was corn (*Zea mays* L.; grain and silage), although in years prior to this experiment Farms 1 and 2 incorporated alfalfa (*Medicago sativa* L.) and soybean [*Glycine max* (L.) Merr.], respectively, into their crop rotation. At each farm, we established two (Farm 1) to three (Farms 2–4) paired cover crop and no-cover-crop plots. All paired plots were within 10 m of each other. Table 1 provides additional information on previous crops, general location, and initial soil characteristics for each farm. Supplemental Tables S1 and S2 provide details for each farm related to weather conditions and management history, respectively.

Cover crop treatments were established in September 2014, with the entire field initially planted to winter cover crops. Cover cropping regimes varied in both planting date and cover crop composition (Table 2). At Farm 1, the cover crop was planted by direct seeding with a no-till grain drill into corn silage residue. Similarly, a no-till grain drill was used to plant cover crops at Farm 2 into soybean stubble and at Farms 3 and 4 into corn stover. The no-cover-crop plots were established within 2 wk of cover crop emergence by treating 80-m^2 plots (~8.9 × 8.9 m) with glyphosate to

Table 1. Site locations and initial soil characteristics of the four farms used in this study. Previous crop denotes the crop planted prior to the planting of cover crops.

Farm ID	Location	Soil series	Previous crop	pH†	P‡	K‡	Ca‡	Mg‡	Zn‡	Mn‡	Cu‡	Fe‡	B‡	CEC§	Soil C¶	Soil N¶
					mg kg^{-1}									cmol$_c$ kg^{-1}	g kg dry wt. soil^{-1}	
1	38°22′ N, 78°52′ W	Frederick silt loam	corn silage	7.0	79	141	1343	163	8	16	1.4	4	1.1	8.4	20.4	1.6
2	38°16′ N, 77°48′ W	Nason silt loam	soybean	6.6	25	136	996	37	2	21	1.3	39	0.3	5.8	16.8	1.1
3	38°21′ N, 79° 1′ W	Frederick silt loam	corn	6.6	93	225	1205	115	8	16	2.2	6	0.7	7.8	20.9	1.6
4	38° 9′ N, 78°56′ W	Edom silt loam	corn	6.0	79	98	1044	130	5	8	2.7	7	0.5	7.7	21.1	1.5

† pH, 1:1 soil/water.

‡ Mehlich I.

§ CEC, Mehlich-I estimate from sum of cations.

¶ Determined using an NA1500 CHN analyzer (Carlo Erba).

Table 2. Soil and cover crop information for the farm sites measured in this study. Cover composition refers to the composition of seeds in the planted mix; seeding rate for each cover crop species is given in the order presented for cover composition; plant date refers to when cover crops were planted; biomass is cover crop dry mass; and biomass C and N refer to the percentage of carbon and nitrogen associated with cover crop biomass.

Farm ID	Replicate plot	Treatment	WHC†	GVM‡	pH	Cover composition	Seeding rate	Plant date, 2014	Sampling date, 2015	Biomass	Biomass C	Biomass N
			g kg^{-1}				kg ha^{-1}			kg m^{-2}		
1	A	cover	269.8	160.3	7.17	triticale, vetch, crimson clover, radish	125, 11, 11, 2	15 Sept.	11 Apr.	8.18	3.24	0.12
1	B	cover	319.5	172.6	6.79	triticale, vetch, crimson clover, radish	125, 11, 11, 2	15 Sept.	11 Apr.	6.89	2.54	0.12
2	A	cover	279.1	225.9	7.29	barley, crimson clover, radish	108, 11, 2	27 Sept.	11 Apr.	0.75	0.29	0.01
2	B	cover	235.4	188.8	7.39	barley, crimson clover, radish	108, 11, 2	27 Sept.	11 Apr.	0.86	0.33	0.01
2	C	cover	247.1	168.9	6.55	barley, crimson clover, radish	108, 11, 2	27 Sept.	11 Apr.	0.75	0.29	0.01
3	A	cover	350.6	200.7	6.69	rye, crimson clover, radish	125, 17, 2	25 Sept.	11 Apr.	7.32	2.86	0.12
3	B	cover	277.5	190.1	6.79	rye, crimson clover, radish	125, 17, 2	25 Sept.	11 Apr.	6.13	2.43	0.10
3	C	cover	302.6	159.9	6.77	rye, crimson clover, radish	125, 17, 2	25 Sept.	11 Apr.	6.13	2.49	0.10
4	A	cover	331.5	292.5	6.86	triticale, vetch	125, 22	20 Sept.	8 Apr.	5.16	2.09	0.12
4	B	cover	280.5	270.1	7.04	triticale, vetch	125, 22	20 Sept.	8 Apr.	6.03	2.45	0.16
4	C	cover	332.3	300.8	6.86	triticale, vetch	125, 22	20 Sept.	8 Apr.	6.13	2.48	0.16
1	A	no cover	238.2	188.0	6.88	na	na	na	11 Apr.	na	na	na
1	B	no cover	280.4	200.6	6.72	na	na	na	11 Apr.	na	na	na
2	A	no cover	247.8	172.9	6.71	na	na	na	11 Apr.	na	na	na
2	B	no cover	222.0	175.9	7.05	na	na	na	11 Apr.	na	na	na
2	C	no cover	237.3	187.7	6.18	na	na	na	11 Apr.	na	na	na
3	A	no cover	322.1	206.9	6.72	na	na	na	11 Apr.	na	na	na
3	B	no cover	290.1	221.6	6.44	na	na	na	11 Apr.	na	na	na
3	C	no cover	266.3	198.3	6.33	na	na	na	11 Apr.	na	na	na
4	A	no cover	301.9	274.9	6.45	na	na	na	8 Apr.	na	na	na
4	B	no cover	292.2	276.4	6.27	na	na	na	8 Apr.	na	na	na
4	C	no cover	315.0	263.3	6.44	na	na	na	8 Apr.	na	na	na

† Water holding capacity.

‡ Gravimetric soil moisture.

terminate the cover crop in either two or three representative areas (i.e., different fields or locations with differing topography) of each farm. Cover-crop plots were identified as 80-m^2 plots adjacent to the no-cover-crop plots. Although we recognize the potential that glyphosate could influence soil properties, recent research indicates that such effects are subtle and outweighed by management and environmental factors (Schlatter et al., 2017).

In early April 2015, just prior to the producer terminating cover crops in the entire field, aboveground plant biomass samples (i.e., cover crop biomass) were collected from a single, randomly identified 1-m^2 area adjacent to each no cover area by hand clipping at soil level. Dry plant biomass samples were dried at 60°C for 48 h in a forced-air oven and ground to pass a 2-mm screen with a Wiley sample mill (Thomas Scientific), and total C and N were determined via dry combustion (VarioMax CNS macro elemental analyzer, Elementar).

Soil Characteristics

In early April 2015, 15 soil cores (1.9 cm in diameter) were taken from each 80-m^2 experimental plot to 15 cm depth and composited by thorough mixing. Soils were sieved (4.75 mm), homogenized, and stored at 4°C for subsequent analyses. A subsample of soil was stored at −80°C for DNA extraction and determination of fungal-to-bacterial ratios (see below).

For each of the soil samples, we determined gravimetric soil moisture, 100% water holding capacity (WHC), soil pH, dissolved organic C (DOC), dissolved organic N (DON), NH_4^+, NO_3^-, and mineralizable-C. Gravimetric soil moisture and WHC (soil was first completely saturated and allowed to drain for 2 h; this is roughly equivalent to field capacity) were determined by drying soil at 105°C for 24 h. Soil pH (1:1, Soil/H_2O by volume) was determined using a benchtop meter. Dissolved organic C, DON, NH_4^+, and NO_3^- in soil solution were determined by first shaking soils for 4 h with 0.5 M K_2SO_4. After shaking, the soil solution was filtered before DOC concentrations were determined using a total organic C analyzer (Model 700 Total Organic Carbon Analyzer, Ohio Instruments Corp.). Dissolved organic N and inorganic N pools were quantified on an autoanalyzer (QuikChem 8500 FIA System, Lachat). Although DOC is often considered a labile soil C pool, it can be composed of both simple and complex C compounds (Strickland and Rousk, 2010). Therefore, we also determined the mineralizable-C pool, which is expected to be indicative of the most labile soil C.

The mineralizable-C pool can be driven by changes in inputs of simple C compounds, likely derived from root exudates (Strickland et al., 2015b). The mineralizable-C pool was determined by maintaining soils at 20°C and 65% WHC for 30 d, with periodic determinations of respiration rates (five measurements across the 30-d period). Using a static incubation procedure, respiration rates were determined via infrared gas analysis of headspace CO_2 concentrations using an infrared gas analyzer (Model LI-7000, Li-Cor Biosciences). Total mineralizable-C was estimated via integration over the 30-d period.

Soil Microbial Community Characteristics

To assess soil microbial community structure and function, we determined microbial biomass C and N, active microbial biomass, catabolic profiles, and fungal-to-bacterial dominance. Microbial biomass C and N were determined using modified chloroform fumigation extraction (CFE) (Fierer et al., 2003). Microbial biomass C and N were determined as the flush of DOC or DON (quantified as described above), respectively, after fumigation. Determining microbial biomass via CFE allowed us to estimate total microbial biomass as well as biomass C/N ratios.

Given the short time frame of this experiment, biomass determined via CFE (i.e., total biomass) may change relatively little; however, active biomass, determined via substrate-induced respiration (SIR), is likely to be more responsive (Ulyshen et al., 2017). We used the SIR method described by Fierer et al. (2003). Briefly, soil slurries were incubated, after a 1-h pre-incubation, with excess C substrate (i.e., autolyzed yeast) for 4 h at 20°C. After this incubation, headspace CO_2 concentrations were determined as described for mineralizable-C.

Microbial community function was assessed via catabolic response profiles (Degens and Harris, 1997; Strickland et al., 2017). This method allowed us to determine substrate-utilization patterns for soil microbial communities between plots with and without cover crops. Specifically, we amended 4 g dry weight equivalent soil with 8-mL solutions (pH adjusted to 6 with NaOH or HCl prior to addition) of glucose, sucrose, glycine, oxalic acid, citric acid, chitin, and cellulose (27, 51, 2, 18, 38, 96, and 96 g of substrate kg $soil^{-1}$, respectively). Resulting soil slurries, except those containing chitin or cellulose, were preincubated for 1 h with shaking and then incubated for an additional 4 h at 20°C. Slurries containing chitin or cellulose were incubated for 24 h after the initial 1-h preincubation. After the incubation, headspace CO_2 concentrations were determined as described for mineralizable-C and SIR.

Fungal-to-bacterial dominance is often considered a soil health indicator, with fungal dominance expected to equate to greater soil health (Bardgett and McAlister, 1999; Hendrix et al., 1986; Strickland and Rousk, 2010). We determined fungal-to-bacterial dominance via quantitative polymerase chain reaction using the method described in Fierer et al. (2005). Total DNA was extracted from ~0.25 g soil using the MoBio PowerSoil Soil DNA isolation kit (MoBio Laboratories). Total bacterial abundance was estimated by quantifying 16S copies using the Eub338/Eub518 primer pair, and fungal abundance was assessed as internal transcribed spacer copy numbers using the ITS1f/5.8s primers. Each 25-mL reaction contained 12.5 μL iTaq Universal SYBR Green Supermix (Bio-Rad, Hercules), 5 μL PCR water (MoBio Laboratories), 1.25 μL of each of the primers (10 μM), and 5 μL of isolated DNA as template. Conditions were 15 min at 95°C, followed by 40 cycles of 95°C for 1 min, 53°C for 30 s, and 72°C for 1 min. Standards were made from 10-fold dilution series of plasmids containing cloned target regions. Sample data were only considered acceptable if standard curve $R^2 > 0.99$ and efficiency (E) was $0.9 < E < 1.1$ for each run.

Statistical Analyses

To assess differences in response variables (i.e., soil pH, soil C and N pools, CFE microbial biomass, SIR microbial biomass, and fungal-to-bacterial dominance) under the presence or absence of cover crops, we used linear mixed effects models assigning cover crop treatment as the independent variable. Farm identity was treated as a random effect, and farm-specific replicates were nested within farm identity, allowing us to account for pseudoreplication of paired plots associated with each farm. Linear mixed effects models allow us to analyze pseudoreplicated data while reducing the likelihood of committing a Type II error (Chaves and Chaves, 2010). Using Euclidean distance, the effect of cover

crops on catabolic profiles was assessed via permutation multivariate ANOVA. Relationships between cover crop biomass and soil and microbial community characteristics were assessed via linear regression. Linear mixed effects models and linear regression were conducted in R version 3.2.2. Permutation multivariate ANOVA was conducted in Primer (Anderson et al., 2008). Significance is considered at $P < 0.05$, and marginal significance is considered at $P < 0.10$.

RESULTS

Soil Characteristics

Cover crop plots had a mean total plant biomass of 4.94 ± 0.84 kg m^{-2}, which consisted of 39.41 ± 0.36% C and 1.92 ± 0.13% N, at the time of sampling. Plots containing no cover crops had sparse to no plant biomass. Mean soil gravimetric moisture content at 100% WHC of the soil increased from 273.9 ± 10.2 (mean ± 1 SE) to 293.3 ± 11.2 g kg^{-1} with cover cropping ($F_{1,10}$ = 12.6; $P < 0.01$). Mean soil pH also increased with cover cropping from 6.56 ± 0.08 to 6.92 ± 0.08 ($F_{1,10}$ = 12.6; $P < 0.001$).

How cover crops affect soil C and N pools was of particular interest in this study. We observed that cover crops increased soil NH_4^+ by 64% from 1.6 to 2.7 mg NH_4^+ kg^{-1} dry weight soil ($F_{1,10}$ = 13.9; $P < 0.01$) (Fig. 1A), whereas soil NO_3^- decreased with cover cropping by 30% from 21 to 14 mg NO_3^- kg^{-1} dry weight soil ($F_{1,10}$ = 6.5; $P < 0.05$) (Fig. 1B). Soil DON did not differ between cover crops and no cover crops ($F_{1,10}$ = 0.37; P = 0.55). Bioavailable C, estimated by mineralizable C, increased by 37% with cover cropping from 0.17 to 0.23 g CO_2–C kg^{-1} dry weight soil ($F_{1,10}$ = 12.7; $P < 0.01$) (Fig. 1C). The soil DOC pool, like DON, did not differ between cover crops and no cover crops ($F_{1,10}$ = 2.9; P = 0.11).

Soil Microbial Characteristics

For soil microbial catabolic response profiles, we observed no effect of cover crops (pseudo-$F_{1,17}$ = 0.61; P = 0.64) (Supplemental Fig. S2), but farm identity did influence catabolic profiles (pseudo-$F_{1,17}$ = 2.2; $P < 0.05$). Microbial biomass C ($F_{1,10}$ = 1.6; P = 0.24) and N ($F_{1,10}$ = 2.5; P = 0.15) and fungal-to-bacterial dominance ($F_{1,10}$ = 0.38; P = 0.55) were unaffected by cover crops. The only soil microbial characteristic that exhibited a response to cover cropping was SIR microbial biomass, which is an indicator of microbial activity ($F_{1,10}$ = 23.3; $P < 0.001$). Active microbial biomass was 64% greater in plots with cover crops compared with those without (Fig. 1D).

Relationships to Cover Crop Biomass

Although we observed differences in several soil and microbial characteristics, these differences were due simply to the implementation of cover cropping. However, aboveground cover crop biomass across our sites varied from 8.18 to 0.75 kg m^{-2}. Therefore, we investigated whether differences in soil and microbial properties

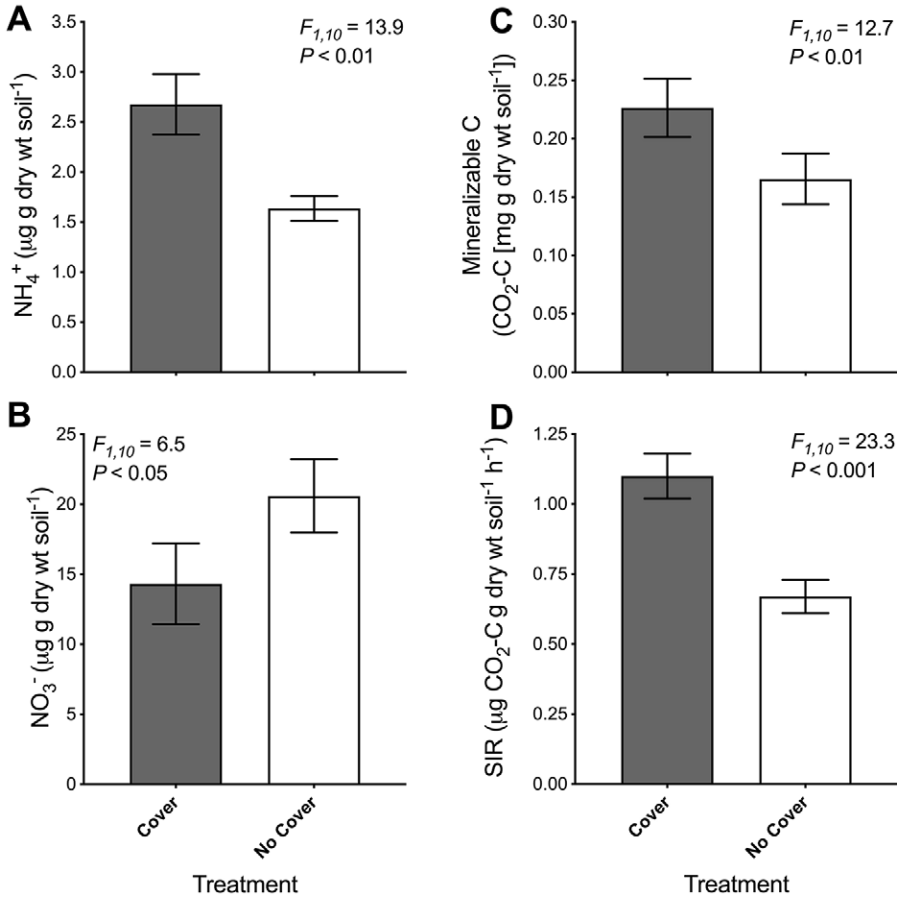

Fig. 1. Effect of the presence (closed bars) or absence (open bars) of cover crops on (A) soil NH_4^+, (B) NO_3^-, (C) mineralizable C, and (D) substrate-induced respiration (SIR) microbial biomass. Mineralizable-C is expected to be a labile, bioavailable pool of soil C that responds rapidly to management. In this instance, mineralizable-C increased by ~37%. Such an increase may have potential implications for soil C sequestration. The SIR biomass is an estimate of active microbial biomass and under cover cropping increased by ~64%. Shown are means ± 1 SE.

were related to standing cover crop biomass because farmers cannot always maximize cover crop yield, even in the same geographic region. Aboveground cover crop biomass did appear to vary by physiographic province: the three farms in the Ridge and Valley (Farms 1, 3, and 4) ranged in biomass from 5.16 to 8.18 kg m^{-2} cover crop biomass, whereas Farm 2 in the Piedmont had much lower yields of 0.75 to 0.86 kg m^{-2}. However, this could also be attributed to differences in management (e.g., manure application) between Farm 2 and the other farms. Of the soil characteristics, only 100% WHC was positively related to cover crop biomass ($y = 0.50x + 20.16$; $F_{1,9} = 5.7$; $P < 0.05$). Of the microbial community characteristics, microbial biomass N, microbial biomass C/N, and SIR biomass were all significantly related to cover crop biomass (Fig. 2). Microbial biomass C was marginally but positively related to cover crop biomass ($F_{1,9} = 4.99$; $P = 0.05$) (Fig. 2A). Microbial biomass N was positively related to cover crop biomass ($F_{1,9} = 12.41$; $P < 0.01$) (Fig. 2B), whereas the microbial biomass C/N ratio was negatively related ($F_{1,9} = 5.7$; $P < 0.05$) (Fig. 2C). Substrate-induced respiration biomass, an indicator of active biomass, was negatively related to cover crop biomass ($F_{1,9} = 6.0$; $P < 0.05$) (Fig. 2D). For microbial catabolic profiles, we found both proportional cellulose ($F_{1,9} = 5.8$; $P < 0.05$) (Fig. 3A) and chitin ($F_{1,9} = 7.0$; $P < 0.05$) (Fig. 3B) respiration were negatively related to cover crop biomass. Proportional glucose respiration was positively related, albeit at marginal statistical significance, to cover crop biomass ($F_{1,9} = 4.2$; $P = 0.07$) (Fig. 3C). All other substrates used in the microbial catabolic profiles were not significantly related to aboveground cover crop biomass.

DISCUSSION

Here we examined soil and microbial community characteristics under management regimes with or without cover crops on working farms in Virginia. This is of particular importance given the paucity of information regarding the efficacy of cover crops on working farms. Actual farms face multiple considerations when implementing a cover crop regime, from factors like weather, which may affect the maximum achievable cover crop biomass, to variation in soil characteristics across fields and farms (Ruis and Blanco-Canqui, 2017). Thus, understanding whether merely implementing cover crops or attempting to maximize cover crop biomass will invoke a positive change in soil and microbial characteristics, along with information on the diversity of cover crop mixes and economic considerations, is needed for farmers to use successful cover crop management regimes. With this in mind, we found that the presence of cover crops, regardless of biomass, had a marked influence on many soil characteristics.

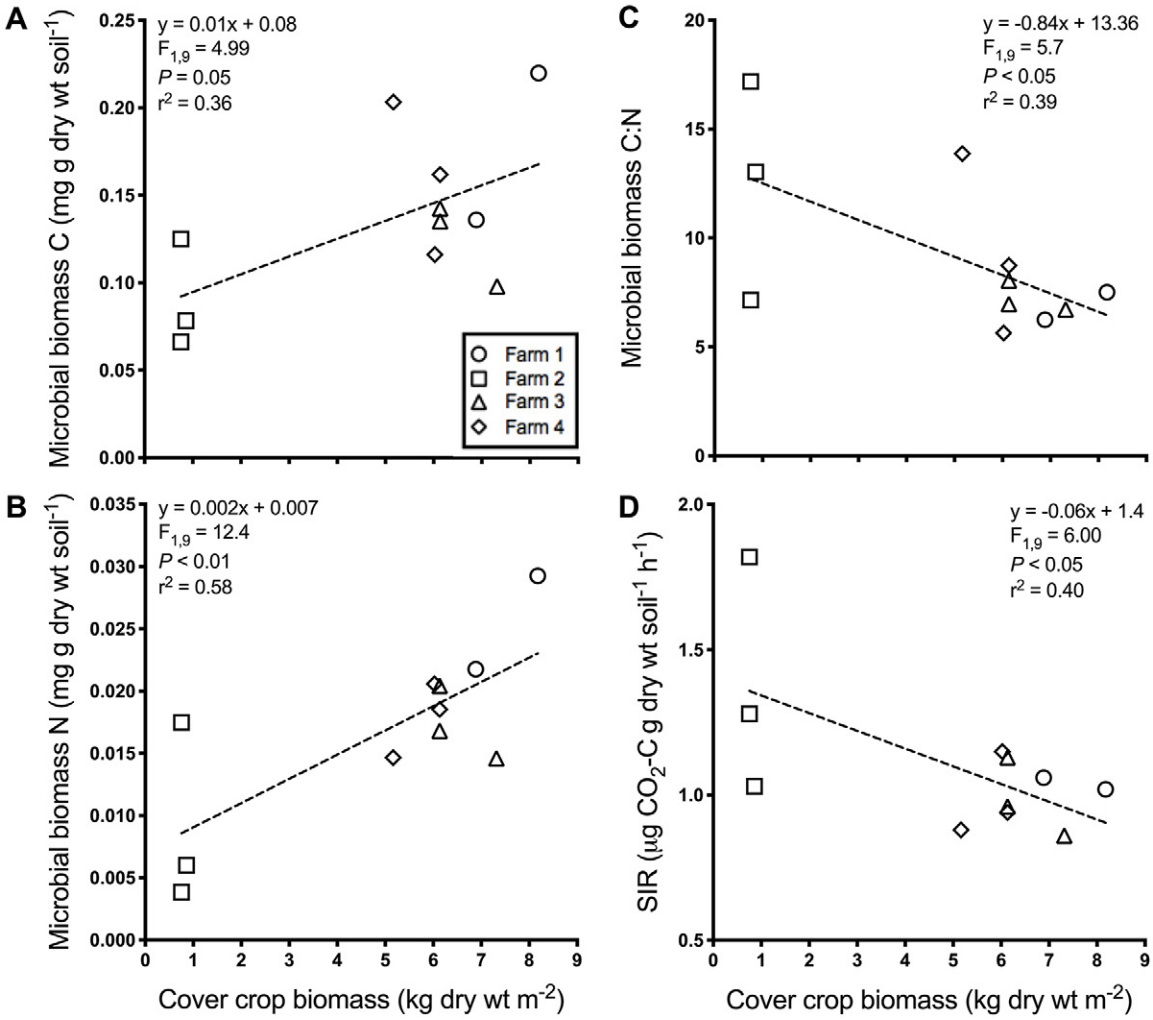

Fig. 2. Relationships between characteristics of the microbial biomass and cover crop dry biomass (kg m^{-2}). (A) A marginally significant ($P = 0.05$) positive relationship was observed for microbial biomass C. (B) A significant positive relationship was observed for microbial biomass N. Significant negative relationships were observed for both (C) microbial biomass C/N and (D) substrate-induced respiration (SIR) microbial biomass. The SIR biomass is expected to be an estimate of the active portion of the microbial biomass.

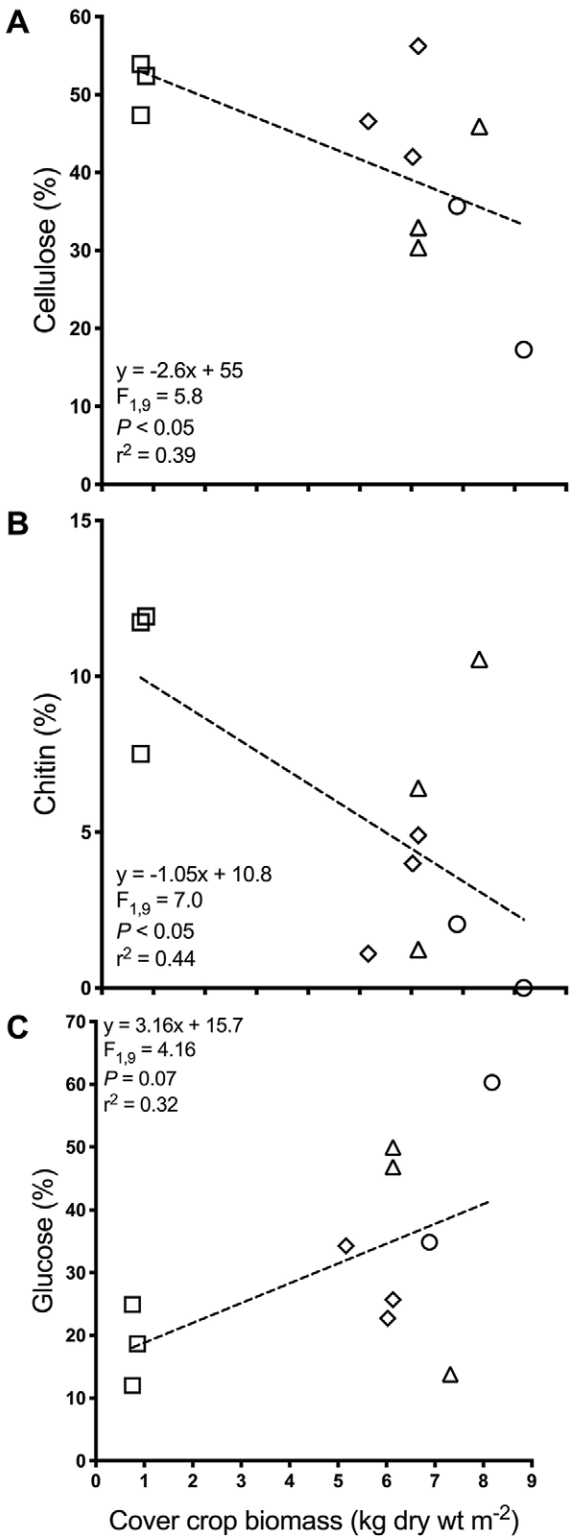

Fig. 3. Relationships between the proportional mineralization of substrates used to determine microbial catabolic profiles and cover crop dry biomass (kg m^{-2}). Significant negative relationships were observed for (A) proportional mineralization of cellulose and (B) proportional mineralization of chitin. (C) For proportional mineralization of glucose, a marginally significant (P = 0.07) positive relationship was observed. Together these results may suggest that, with increasing cover crop biomass, soil microbial communities shift from the mineralization of more recalcitrant substrates toward the mineralization of more labile substrates. Circle, Farm 1; square, Farm 2; triangle, Farm 3; diamond, Farm 4.

In particular, mineralizable-C was ~37% greater—an average change of 0.17 to 0.23 g CO_2–C kg^{-1} dry weight soil—when cover crops were present (Fig. 1C). This is a striking increase in what is considered the bioavailable C pool. The importance of this benefit is critical given the expectation that this pool of C will be preferentially utilized by soil microbes and may ultimately lead to the formation of stable SOM (Bradford et al., 2013; Strickland et al., 2015a). Whereas other studies have shown such increases in soil C pools with cover cropping (Ladoni et al., 2016; Mazzoncini et al., 2011; Poeplau and Don, 2015), ours expands on this knowledge to show that positive increases occur across ranges of cover crop standing biomass and species composition in a relatively short period of time (Table 1).

Not surprisingly, with an increase in bioavailable C, we also observed a significant 64% increase (average change, 0.67–1.10 mg CO_2–C kg^{-1} dry weight soil h^{-1}) in active (i.e., SIR) microbial biomass (Fig. 1D). However, total microbial biomass C and N were unaffected by cover cropping. This suggests that, although the microbial biomass is more active under cover cropping, cover cropping did not lead to an increase in the total microbial biomass pool. One reason may be that one season of winter cover crops in rotation may not be long enough to stimulate an increase in total biomass. Alternatively, the farms used in this study all used no-till management, which may have led to a stable, large standing pool of total microbial biomass that is ultimately unaffected by cover crops. Under no-till management regimes, cover cropping has been shown to have a limited effect on total microbial biomass C (Liebig et al., 2015; Mbuthia et al., 2015), but active microbial biomass (i.e., SIR biomass) has not often been measured. Additionally, the presence of cover crops could have decreased the level of microbial dormancy, potentially via inputs of root exudates, meaning that total biomass remains unchanged but microbial activity increases (Jones and Lennon, 2010). From our data, it appears that, although total biomass may be a product of tillage management, cover cropping may influence the activity of that biomass.

Cover cropping also influenced some soil N pools at the time of sampling. We observed an increase in soil NH_4^+ and a decrease in soil NO_3^- with cover cropping. This shift in N species is potentially driven by preferential plant uptake of NO_3^- and by increased microbial activity leading to greater N mineralization associated with cover crops. With the observed shifts in N pools associated with cover cropping, it seems possible that the implementation of this management regime could reduce N leaching from agricultural soils and associated risks to nearby water resources (Wyland et al., 1996). It is also likely that these shifts in N pools may account for the less acidic pH observed in sites with cover crops (Table 2).

In addition to the effect of the presence versus absence of cover crops, we examined whether variation in standing cover crop biomass across sites influenced soil and microbial community characteristics. Such a relationship is important given the wide range of cover crop biomass observed on different farms in this study but also regarding the constraints (e.g., weather or maximizing the primary crop's growing season) farmers face when implementing a cover crop into their rotation (CTIC, 2017). These types of constraints could explain, in part, the results observed for Farm 2, which had the shortest window of cover crop growth due to a combination of rainfall (Supplemental Table S1) and later planting necessitated by the previous soybean crop, albeit this site differed in several other characteristics (in particular, Farm 2 had not amended manure in

the past 5 yr, whereas the other Farms had) (Table 1). In our results, the variables related to cover crop biomass were generally estimates of microbial biomass and catabolic activity (Fig. 2 and 3). We hypothesize that such variables are most likely to respond rapidly to changes in plant biomass (Bais et al., 2006). For instance, we observed that both microbial biomass C and N tended to increase with increasing cover crop biomass. Additionally, the microbial biomass C/N ratio decreased with increasing cover crop biomass. Cover crop species that vary in biomass C/N ratio may also play a role, particularly when considering microbial biomass N and biomass stoichiometry. That is, all of the farmers in this study incorporated a legume in their cover crop mix, and those farms that had greater cover crop biomass tended to be associated with greater soil microbial biomass N and a lower biomass C/N ratio. This could indicate that microbial communities associated with greater cover crop biomass (specifically leguminous biomass) may be under less nutrient stress and, as such, may conduct less N mining from the existing SOM (Craine et al., 2007). However, more research is necessary, particularly given Farm 2's marked influence on the relationships between cover crop biomass and soil microbial characteristics (Fig. 2 and 3), to explicitly examine potential links between plant and microbial biomass stoichiometry as it relates to cover crop management.

For active (SIR) biomass, we observed a negative relationship with increasing cover crop biomass (Fig. 2C). One potential reason is that with increasing aboveground biomass there is greater competition aboveground and ultimately less allocation of plant-derived C primarily in the form of labile root exudates belowground (Wilson et al., 2018). This may suggest that farmers could use strategies that stimulate microbial activity via mowing or grazing cover crops, reducing aboveground plant competition (Franzluebbers, 2007). By creating a more continuously active microbial community via such active cover crop management, accrual of SOM may increase more rapidly (Wilson et al., 2018). Alternatively, this observation may be due to the composition of cover crops planted, the previous crop, or the underlying soil characteristics associated with the farms used in this study. For instance, the primary driver of the observed negative relationship could be attributed to a single farm (i.e., Farm 2), which differed from the other farms regarding several characteristics, including soils and cropping regimes (Tables 1 and 2; Supplemental Tables S1 and S2). Additionally, Farm 2 was the only farm that did not receive manure in the last 5 yr (Supplemental Table S2). This could also explain why cover cropping led to a strong increase in active microbial biomass. That is, if cover crops were under lower nutrient conditions (i.e., lacked manure inputs), then a greater allocation of plant-derived resources may have been allocated belowground to acquire soil nutrients and thus stimulate microbial activity (Wilson et al., 2018). This suggests that the effect of cover cropping may be context dependent, with the benefits of cover crops mediated by site conditions and history. Further research should be conducted to identify the potential factors that may influence the efficacy of cover cropping across multiple site-years.

We also found relationships between the proportional mineralization of several compounds used in the catabolic profiles to cover crop biomass. We observed that the mineralization of cellulose and chitin (compounds expected to be more recalcitrant) showed negative relationships with cover crop biomass, whereas glucose (a labile compound) mineralization responded positively. This may indicate a shift from microbial utilization of recalcitrant substrates toward more labile substrates. Such a shift could be driven by increased utilization of root exudates or decreased N mining of recalcitrant substrates by the soil microbial community (Craine et al., 2007; Strickland et al., 2015b). In either instance, this suggests the potential for cover cropping to increase SOM formation, particularly if more recalcitrant forms of soil- and plant-derived C are not being mineralized disproportionally. Future research to understand the specific mechanism driving such patterns may lead to better management strategies for cover crops (e.g., by using cover crop species or mixes that maximize root exudation).

Although this study included only four sites measured across a single growing season, we found that the use of cover crops can have a marked effect on several soil and soil microbial community characteristics, both by the mere presence of cover crops and through change in cover crop biomass. Such effects can be realized by farmers even when individual farms are using different cover crop mixes and attain various amounts of cover crop biomass, as was the case in our study. In addition, some of the potential effects (i.e., microbial biomass C and N, active microbial biomass, and catabolic profiles) of cover crops were related to cover crop biomass, which suggests that if farmers are able to maximize biomass, then the beneficial effects of cover crops may also be maximized. However, we suggest that this relationship may be more nuanced and that there is a need to better understand aboveground–belowground linkages, especially across multiple sites and years, along with the influence of plant competition, when suggesting cover crop management regimes.

SUPPLEMENTAL MATERIAL

Supplemental Fig. S1. Representative plots showing the no-cover and cover treatments at the time of sampling for (A) Farm 2, and (B) Farm 4.

Supplemental Fig. S2. Principal coordinates analysis plot for the catabolic response profiles associated with each farm and cover crop treatment. Farms are indicated by different symbols and plots with cover crops are indicated in green while those without cover crops are indicated in brown.

Supplemental Table S1. Monthly average temperature (°C) and monthly precipitation (mm) associated with each farm from September 2014 to April 2015 (i.e., the timeframe of this study).

Supplemental Table S2. Management and yield history associated with each site prior to the initiation of the experiment.

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