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ARTICLE

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Nitrogen services provided by interseeded cover crops in organic corn systems

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Abstract

Cover crops (CC) can be used to reduce soil inorganic N (SIN) losses from agricultural soils. However, challenges exist in establishing CCs after corn (*Zea mays* L.) harvest in regions with a limited window for fall CC growth. One solution to this constraint is to establish a CC by interseeding into a standing corn crop. This experiment aims to assess the effect of interseeding CCs on the N cycle. Within organically managed corn grain and silage systems, we implemented three CC treatments: interseeded annual ryegrass (*Lolium multiflorum* Lam.), postharvest seeded cereal rye (*Secale cereale* L.), and a no-CC fallow. We applied fertilizer at a standard level (336 kg N ha^{-1}) and at a high level (420 kg N ha^{-1}). Silage was harvested in October and grain in November. Corn yields were not affected by CC treatments. In the fall in the silage system, the interseeded treatment had reduced SIN compared with the postharvest seeded treatment. In the spring, both interseeded and postharvest seeded treatments had less deep SIN than fallow in grain and silage. Under high N conditions in the spring, interseeded treatments had greater microbial biomass than all other treatment combinations. Both CC treatments and applied N levels affected microbial taxonomic groups. In terms of N retention services, interseeded CCs do have potential to outperform a postharvest seeded CC with a longer window for unshaded fall growth (as in a silage system) and may perform just as well as a postharvest seeded CC in the spring.

1 | INTRODUCTION

Integrating fall sown cover crops (CC) into agricultural systems can provide a suite of agronomic and environmental benefits, including improved soil quality, increased nutrient retention and reduced losses, improved pest management, and suppression of weeds (Finney et al., 2015; Schipanski et al., 2014; Snapp et al., 2005; Wallace et al., 2017). These CCs fill fallow niches in crop rotations when and where soils are vulnerable to erosion and nutrients are vulnerable to losses via leaching and runoff. Additionally, there is an aboveground–belowground link between CCs and soil microbes that plays a key role in N retention and C storage (de Vries & Bardgett, 2012; Nivelle et al., 2016). Inputs of C and N to the soil, in this case from CC root exudates and CC residues, can affect the size of the soil microbial biomass and the function of the soil microbial community (for example, functions like decomposition, nitrification, N-fixation), both of which have implications in nutrient retention and cycling (Finney et al., 2017; Rankoth et al., 2019).

Abbreviations: CC, cover crop; PLFA, phospholipid fatty acid; RDA, redundancy analysis; rRNA, ribosomal RNA; SIN, soil inorganic nitrogen.

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Furthermore, as the demand for organic grain has increased in the northeastern United States, there has been increased interest among farmers in adopting organic growing practices as this market provides a high-value opportunity (Greene et al., 2017; Roth, 2010). In 2015, U.S. organic sales were estimated at US$43.3 billion by the Organic Trade Association (Greene et al., 2017) and by 2020 reached $61.9 billion (Organic Trade Association, 2021). It is, therefore, imperative to develop organic grain cropping systems that will achieve agronomic and economic goals while maintaining low impacts on the environment. Research evaluating the integration of innovative CC strategies in organic grain systems has potential for significant effects.

A short and highly variable window for fall CC establishment is an issue identified both in the scientific literature and by farmers (Ketterings et al., 2015; Wayman et al., 2017). To address this limitation, CCs can be interseeded into standing cash crops (Baributsa et al., 2008; Brooker et al., 2020; Curran, Hoover, Mirsky, et al., 2018; Groff, 2015; Regan et al., 2020; Wallace et al., 2020). With this strategy, CCs can be established in midsummer, reducing the risk of a failed CC establishment after cash crop harvest because of seasonal or other constraints. Additionally, interseeded CCs provide a longer window of CC growth with potential for maximizing CC benefits.

Recent work on interseeding CCs has established this practice as a viable option for grain farmers. Much of this research has focused on CC seeding rate, seeding method, species selection, and planting timing to ensure CC establishment without competition to the cash crop or effects on grain yield (Abdin et al., 1998; Belfry & Van Eerd, 2016; Brooker et al., 2020; Caswell et al., 2019; Noland et al., 2018; Rusch et al., 2020; Youngerman et al., 2018). The provisioning of agronomic and environmental benefits from an interseeded CC will vary depending on CC species traits. For example, cultivars of grasses and brassicas with deep roots can act as N scavengers (Dean & Weil, 2009), whereas N-fixing legumes can add N to a cropping system (Ranells & Wagger, 1996). Known traits of CC species can be used to manage N services at specific times in a crop rotation (Kaye et al., 2019). Several CC species, including annual ryegrass (*Lolium multiflorum* Lam.), have been identified as optimum for interseeding in corn (*Zea mays* L.) grain and silage systems, which are common cropping systems in the northeastern United States and across the Midwest. Multiple studies have found that interseeding an annual ryegrass CC during the growth stages V4–V7 in corn will successfully establish the CC without inducing competition to the corn crop (Brooker et al., 2020; Caswell et al., 2019), achieving successful niche complementarity.

Whereas the practice of interseeding CCs is not new (e.g., Dickey, 1947), to our knowledge, this experiment is the first to manipulate soil N status in order to test the effect of interseeded CCs and soil microbes on N retention in an organic system. Cover crops can retain labile soil N, both protecting N from losses to the environment and storing N for later use by a growing cash crop (Finney et al., 2015; Gardner & Drinkwater, 2009; Ketterings et al., 2015). Especially in corn systems that require high N inputs, N management for increased retention and reduced losses without affecting yields is crucial.

Core Ideas

- Interseeded annual ryegrass CC can retain SIN in corn systems.
- In late fall in a corn silage system, interseeded CC treatments had reduced surface SIN.
- In spring, interseeded CC and postharvest cereal rye CC treatments had less deep SIN than a no-CC treatment.
- Soil microbial biomass was greatest in interseeded systems under high SIN conditions.
- Maximizing time for unshaded fall interseeded CC growth may increase N retention in corn systems.

Other recent work with interseeded CCs has included soil N measurements, which has added to our understanding of the effects of this CC strategy on N dynamics in corn systems. Some experiments found reduced soil N in both the fall and the spring during CC growth: Grabber et al. (2014) found an interseeded annual ryegrass system to have 50% lower residual fall nitrate concentrations in the top 30 cm of soil than a fall seeded rye or a no-CC control, and Noland et al. (2018) found that interseeded CCs reduced spring soil nitrate. In organic trials with interseeded annual ryegrass–radish (*Raphanus sativus* L.) mixtures, Wallace et al. (2020) found different N retention patterns based on latitude and the proportion of winter-killed radish in the interseeded CC stand. The nascent understanding offered by these studies is that interseeded CCs can reduce soil N concentrations. However, if this practice is to become widespread, a more thorough understanding of plant and microbial N interactions is needed to predict how interseeded CCs affect the N cycle across different management conditions.

The goal of this research was to experimentally test CC interseeding as a strategy to reduce nitrate leaching in organic corn grain and silage production. We hypothesized that interseeded CCs would reduce residual labile soil N, thus reducing N losses via both N retention in the CC biomass and N retention in the associated soil microbial pool. To evaluate this, we measured N retention in the CC biomass, estimated potential soil N losses via leaching, and evaluated dynamics in microbial composition and biomass as they relate to N services. This

experiment directly ties CC interseeding effects to N retention services, shifts in microbial composition and biomass, and crop yields in fields with a range of N fertilizer inputs.

2 | MATERIALS AND METHODS

2.1 | Experimental design

This experiment was conducted at the Russell E. Larson Agricultural Research Center in central Pennsylvania, USA (40°43′ N, 77°56′ W) in 2017–2018. Crops were managed according to USDA organic standards throughout the experiment, though the land was not certified organic. The research station is in Zone 6b of the USDA Plant Hardiness Zones and the soil is a well-drained Hagerstown silt loam (fine, mixed, semiactive, mesic Typic Hapludalf). The annual precipitation for 2017 at this site was 1,172 mm (NRCS Rock Springs, PA weather station). This site has an average annual precipitation of 1,020 mm and an average monthly temperature range of 3–22 °C (Xia et al., 2012).

A corn grain system and a corn silage system were evaluated side-by-side in separate factorial experiments, each with a randomized block design and four replicates. The corn grain system included two factors (a CC factor and a N application factor) assigned randomly in a split plot design with CC treatments applied at the subplot level. The corn silage system included only the CC factor. All experimental plots were managed consistently in terms of timing and methods of tillage, planting, and cultivation. Each plot was 12 × 6 m.

The CC factor included three treatments: (a) a no-CC treatment (fallow), (b) a cereal rye (*Secale cereale* L. 'Aroostook') CC established after corn harvest (postharvest) and seeded at 135 kg seed ha^{-1}, and (c) an interseeded 'KB Supreme' annual ryegrass CC treatment seeded at 23 kg seed ha^{-1}. Interseeded plots were drill seeded after final cultivation when corn was at the V5–V6 growth stage. Species selection for the interseeded treatment was based on prior work at this site (Isbell et al., 2021) and other interseeding trials (Caswell et al., 2019; Curran, Hoover, Roth, et al., 2018; Wallace et al., 2020). The seeding rate of the interseeded CC was determined from Penn State Extension publications (Roth et al., 2017).

The N application factor included two levels of fertilizer application: (a) fertilizer to meet the N demands of the corn crop (standard N, 336 kg N ha^{-1}), and (b) fertilizer to exceed the N demands of the corn crop (high N, 420 kg N ha^{-1}). The silage system included only the high N treatment. In the standard N treatment, fertilizer was applied in a split application: half before corn planting and half at corn growth stage V5–V6 (just before interseeding of CCs). In the high N treatment, fertilizer was applied in three applications: at planting, at corn growth stage V5–V6, and at harvest. The high N treatment was used to simulate a system with high enough N that there would be residual labile N in the soil at the time of harvest, which would then be susceptible to losses to the environment. This was done to allow better assessment of the differences in the fate of residual soil N among CC treatments. The first fertilizer application before corn seeding was liquid dairy manure at a rate of 168 kg N ha^{-1}, the second application at last cultivation was pelletized poultry litter (Perdue Ag Microstart 3–2–3) at a rate of 168 kg N ha^{-1}, and the final application in the high N treatment was a soymeal-based fertilizer (McGeary 8–1–1) that was spread by hand at a rate of 84 kg N ha^{-1}. Fertilizer application rates were determined using guidelines in the Penn State Organic Production Guide (White et al., 2015) along with information from the manure analysis reports.

Before corn planting, the site was chisel plowed, disked, and cultimulched. Corn grain variety, seeding rates, planting date, and cultivation followed the Penn State Organic Crop Production Guide (White et al., 2015). Corn (*Zea mays* v. 'Master's Choice 4050') was planted on 2 June 2017 at 81,510 seeds ha^{-1}. Weeds were controlled via mechanical cultivation four times approximately once per week, with the final cultivation just before interseeding in July. Corn silage was harvested 20 Oct. 2017 and corn grain was harvested 17 Nov. 2017.

On 12 July 2017, annual ryegrass was interseeded with the InterSeeder Cover Crop Planter (Interseeder Technologies). This is a 2-row high-clearance grain drill designed to seed three rows on 19-cm spacing between 76-cm cash crop rows. In the interseeded CC treatments, three rows of annual ryegrass were drill-seeded between each corn row. In the postharvest CC treatments, cereal rye was drill seeded into the silage system after corn harvest in October and in the grain system after corn harvest in November.

2.2 | Analysis of soil inorganic N

To estimate the effects of treatments on N retention and leaching, we measured the concentration of extractable soil inorganic N (SIN) in surface soils to document when surface N became available for nitrification or nitrate leaching. We took surface soil samples in mid-October, late October, December, and the following June. One soil sample was collected from each plot by combining six 0.10-m depth by 0.02-m diameter cores. After homogenizing each sample, one fresh 20-g subsample was extracted for inorganic N with 100 ml of 2-M KCl. Following 1 h of shaking, extracts were filtered through Whatman 1 filter paper and frozen until further analysis. Extracts were analyzed colorimetrically for ammonium and nitrate concentrations (Doane & Horwath, 2003; Sims et al., 1995). Soil inorganic N represents the sum of extractable ammonium N and extractable nitrate N. Additional fresh 10-g subsamples were dried at 105 °C for

48 h and weighed to calculate gravimetric water content. Rock content was estimated by wet sieving each sample with a 2-mm mesh sieve, considering particles larger than 2 mm as rocks. Sample weight was corrected for soil gravimetric water content and rock content. Additionally, a subsample of homogenized soil from the first soil sampling event was submitted to The Pennsylvania State University's Agricultural Analytical Services Lab in State College, PA, for standard soil fertility testing.

At the end of the experiment in the spring (June), deep soil cores to 80 cm were collected. Two cores were collected from each plot, separated into 20-m sections, and extracted and analyzed as detailed above. Previous research at this site has shown that spring concentrations of SIN at depth are positively correlated with leaching through the soil profile (Kaye et al., 2019).

2.3 | Analysis of aboveground plant biomass

To quantify differences in CC–N uptake, N content in CC biomass samples was measured throughout the experiment. The first CC biomass sample was taken in October. The second was taken in late November to represent peak fall biomass (before a hard frost), and the last was taken in June to represent peak spring biomass (before a tillage-termination event). At each sampling event, we clipped the aboveground biomass of both CCs and weeds from two 0.5-m^2 quadrats in each plot. We then separated the biomass into CCs and weeds, oven-dried the biomass, and recorded the dry weight. Cover crop biomass was ground into a fine powder and a subsample was rolled into a tin capsule. It was then analyzed for C and N content by dry combustion analysis (CHNS-O CE Instruments Thermo Electron Corp Elemental Analyzer EA 1110 with thermal conductivity detector) (Matejovic, 2003). The same process was used to determine N concentration in corn ear leaves collected in August. Nine corn ear leaves were collected per plot. Corn was harvested by combine at the plot level. Grain was adjusted for moisture content at 15.5%.

2.4 | Analysis of microbial respiration and potentially mineralizable N

To estimate differences in the nutrient cycling capacity of soil microbial communities, we used a laboratory incubation. We used subsamples from soils collected at 10-cm depth in December 2017 and June 2018 that had been dried and stored for later incubation. Each soil sample was incubated in triplicate. Incubations used 10 g of dry soil that were rewet in a serum bottle with 6 ml of deionized water to achieve estimated field water holding capacity at 50% filled pore space. After rewetting, a 24-h period was allowed to pass for the soil to return to basal respiration before the bottle was capped. Soil was then incubated at room temperature for 7 d, after which the headspace gas was sampled and measured using a Li-Cor LI-7000 (Li-Cor) to analyze CO_2. We also measured the initial SIN of a soil subsample and the SIN in each incubated sample at the end of the incubation via extraction methods as previously detailed. Results from similar methods have been shown to be directly proportional to microbial biomass and tied to potential C and N mineralization (Franzluebbers et al., 2000).

2.5 | Analysis of soil microbial biomass and composition

Microbial biomass and composition were evaluated with a phospholipid fatty acid (PLFA) analysis. This analysis was performed on soils from the December 2017 sampling and the June 2018 sampling. Analysis was done by Microbial ID, Inc., on frozen samples using a high-throughput method (Buyer & Sasser, 2012). Phospholipid fatty acid analysis provides a proxy for total microbial biomass and the biomass of taxonomically coarse groups of microbes. Total PLFAs, which represent an index of living microbial biomass, were the sum of all PLFA markers. Biomarker fatty acids that originate from broad microbial groups were used to calculate the biomass of each group in each sample (Supplemental Table S2). Fatty acids that were ubiquitous and could not be categorized into a single group were not used in microbial group assignment, so the total PLFA biomass in a sample does not necessarily equal the sum of the biomass from all coarse groups in a sample.

Finally, to further parse effects of interseeding and soil N status on bacterial composition, we used high-throughput 16S ribosomal RNA (rRNA) gene amplicon sequencing to examine the taxonomic profiles of bulk soil under each treatment in the fall (December 2017) and spring (June 2018). DNA was extracted from 0.25 to 0.30 g of each soil sample with the Machery-Nagel soil extraction kit (MACHEREY-NAGEL GmbH & Co. KG). Universal 16S rRNA gene primers 515F/806R (Apprill et al., 2015; Parada et al., 2016) were used for initial amplification, targeting amplification of the V4 region of the 16S rRNA gene to detect both bacteria and archaea. Amplicon cleaning, barcode attachment, and polymerase chain reaction cycling conditions were carried out as detailed in Howard et al., 2017. After pooling of samples, sequencing was performed using an Illumina MiSeq with the 2×250 cycle v2 kit at the Cornell Genomics Facility at Ithaca, NY and sequencing read files were generated using the Illumina pipeline software v2.18. Raw reads have been uploaded to the NCBI Sequence Read Archive and are available under project number PRJNA751608.

Sequences were processed in R (R Core Team, 2018) using the DADA2 pipeline (Callahan et al., 2016) to assign

amplicon sequence variants, with additional downstream processing using *phyloseq* (McMurdie & Holmes, 2013). Silva version 138 was used to assign taxonomy with training data formatted for DADA2 (McLaren, 2020; Quast et al., 2013). A total of 39,615 input sequences were used after filtering, merging, and removal of chimeras. One sample was eliminated from downstream analyses because of a low number of sequences; the cutoff used for eliminating samples was a minimum of 2,000 sequences, and the one sample eliminated had 178 sequences.

2.6 | Statistical analysis

Statistical analyses were completed in the software environment R (R Core Team, 2018). To evaluate the effect of CC and N application factors on total SIN over the growing seasons, we used a mixed-model repeated-measures approach with the statistical package *lme4* (Bates et al., 2015). Total SIN was log-transformed, with CC treatment, N application level (in the grain system), sampling date, and their interactions as fixed effects and block as a random effect. Post hoc pairwise comparisons of least squared means were performed with a Bonferroni correction for multiple pairwise tests ($\alpha = .05$). For deep soil SIN, aboveground biomass, biomass N, corn yields, CO_2 respired, and PLFA biomasses, mixed-models were used with CC treatment, N application level (in the grain system), sampling date, and their interactions as fixed effects and block as a random effect. Transformations were applied to meet normality assumptions where needed. The ANOVAs were considered significant at $P < .05$.

Additionally, redundancy analysis (RDA) was performed with the package *vegan* (Oksanen et al., 2020) on PLFA groups (Supplemental Table S2) and total PLFA biomass. The statistical significance of the RDA and the significance of canonical axes were tested with permutation analyses. This model used total SIN as an explanatory variable as this measurement co-occurred with the PLFA soil sampling. In corn grain, sampling date, CC treatment, and N application level were also included as explanatory variables, and in corn silage only sampling date and CC treatment were included as explanatory variables. Block was included as a conditional variable in both models.

After taxonomy was assigned to 16S rRNA gene amplicon sequences, PERMANOVA was used to test for differences among treatments using the 'adonis' function in the package *vegan* (Oksanen et al., 2020). Additionally, ANOVAs with post hoc Tukey's HSD tests ($\alpha = .05$) were used to determine differences in relative abundances of bacteria at the phylum level and at the class level within the phyla Proteobacteria. Taxonomy was visualized with krona plots (Ondov et al., 2011). Differences in community structure were estimated using Bray–Curtis dissimilarity and visualized with principal coordinates analysis (Supplemental Figure S1). The PICRUSt2 algorithm was used for prediction of metagenomic functions of the microbial community from the 16s rRNA amplicon sequence data (Douglas et al., 2020).

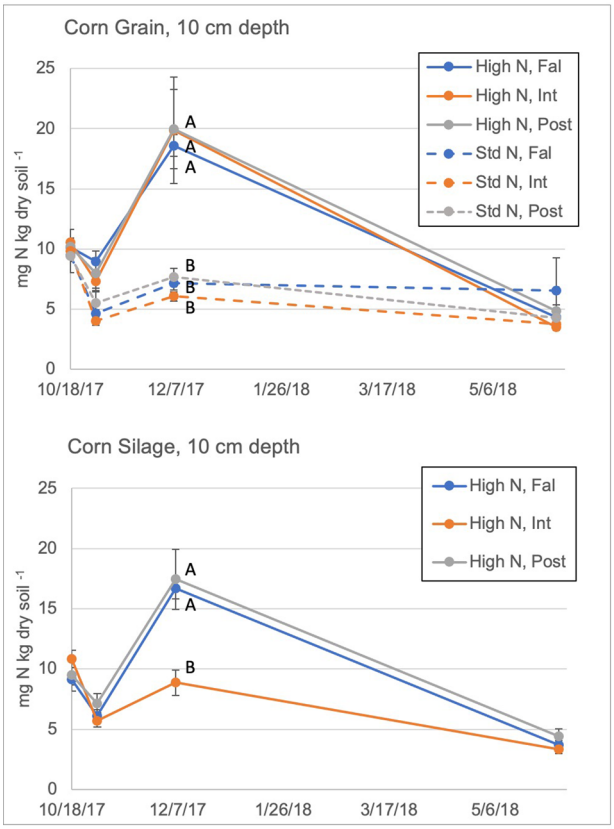


FIGURE 1 Extractable soil inorganic N (mg N kg dry soil^{-1}) at a depth of 10 cm during the cover crop growing season in corn grain and corn silage systems. Solid lines represent the high N treatment (420 kg N ha^{-1}), which included an additional application of N in mid fall. Dashed lines represent the standard N treatment (336 kg N ha^{-1}). Error bars represent SE. Letters indicate significant differences at $P < .05$. Fal, fallow; Int, interseeded annual ryegrass; Post, postharvest seeded cereal rye

3 | RESULTS

3.1 | Soil inorganic N

In the grain system, we observed an expected spike in SIN at the December sampling date, resulting in significant differences between the high N treatments and standard N treatments (Figure 1). By the following June, average SIN had decreased in all treatments. In the silage system, we measured a similar pattern of a spike in SIN in December (Figure 1). However, the interseeded treatment had less SIN than the fallow treatment ($P = .0012$) and less SIN than the postharvest CC treatment ($P = .003$). These differences in the

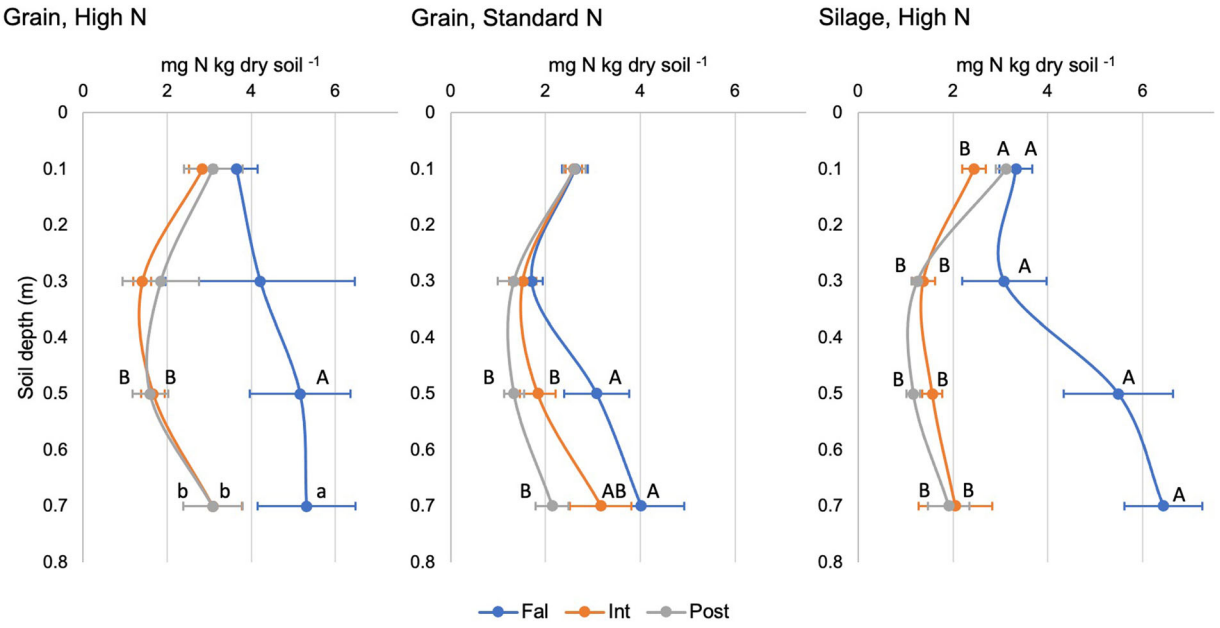


FIGURE 2 Extractable inorganic soil N (mg N kg dry soil^{-1}) from deep soil cores to 0.8 m in corn grain and corn silage systems. Soil samples were taken in the spring of 2018 in corn grain and silage systems before cover crop termination when cover crops were at peak biomass. Two levels of N application were included in the grain system (high N and standard N). The *y* axis represents the average of 20-cm increments of soil depth, 0–0.2 m, 0.2–0.4 m, 0.4–0.6 m, and 0.6–0.8 m. Error bars represent SE. Capital letters indicate statistically significant differences among cover crop treatments within each depth at $P < .05$. Lowercase letters represent significant differences at $P < .10$. Fal, fallow; Int, interseeded annual ryegrass; Post, postharvest seeded cereal rye

silage December SIN were driven by nitrate (NO_3^-) concentrations: interseeded systems had 4.1 mg NO_3–N kg dry soil^{-1} (SE 2.4), postharvest systems had 10.6 mg NO_3–N kg dry soil^{-1} (SE 2.5) and fallow had 12.6 mg NO_3–N kg dry soil^{-1} (SE 0.94).

In the grain system, despite there being no differences in average spring SIN in the shallow surface soil, we did find differences throughout the deeper soil profile (Figure 2). At all N levels, there was greater SIN in the fallow treatment than in both CC treatments at a depth of 0.5 m ($P < .001$). This trend persisted at a depth of 0.7 m ($P < .10$). Soil inorganic N at depth in the silage system closely resembled the pattern in the grain high N system. However, in the silage system the pattern was more pronounced with the two CC treatments having significantly less SIN throughout the soil profile, not just at the depth of 0.5 m. Even in the surface soil (0.1 m), the interseeded treatment had less SIN than the fallow treatment ($P = .013$) and the post treatment ($P = .057$). At 0.3 m, SIN in interseeded and postharvest treatments diverged from the fallow treatment ($P = .014$ and $P = .008$, respectively), and remained at a lower SIN concentration at 0.5 m and 0.7 m ($p < .001$). At 0.7 m, the fallow treatment had an average SIN of 6.4 mg N kg^{-1} dry soil, representing the highest SIN among all soil depths, whereas the interseeded and postharvest treatments were drawn down to 2.1 and 1.9 mg N kg^{-1} dry soil. This was a more than threefold reduction of SIN.

3.2 | Aboveground plant biomass

There were no differences in interseeded annual ryegrass growth rates between the high N and standard N treatments in corn grain based on two sampling events (October and November) (Table 1). This was expected as both treatments had the same amount of N applied at the time of sample collection (the high N treatment received its third application of fertilizer in November at grain harvest). Weed biomass also did not statistically differ between high and low N treatments; however, there was very high variability in weed biomass across plots. In the silage system, the high N treatment received its third application of fertilizer in October at silage harvest, and it is notable that the annual ryegrass CC biomass reached an average of 401 kg ha^{-1} by November (Table 1).

In the corn grain system, there was not a difference in peak spring biomass of the interseeded annual ryegrass between the high N and standard N treatments (Figure 3); however, the postharvest seeded cereal rye resulted in significantly more biomass than the interseeded annual ryegrass ($P < .001$). Similarly, the silage system yielded a significantly lower interseeded annual ryegrass biomass compared with the postharvest cereal rye biomass ($P < .001$). Whereas it does appear that the silage system generated more spring biomass than the grain system in both CC treatments, this was not a

TABLE 1 Mean dry aboveground plant biomass in fall of 2017 in the interseeded treatment

Corn system	Plant type	Nitrogen application level	October mean dry biomass	November mean dry biomass
			kg ha^{-1}	
Grain	Annual ryegrass	High	131 (22.9)	298 (36.4)
	Annual ryegrass	Standard	97.2 (6.9)	266 (35.4)
	Weeds	High	171(92.8)	51.2 (23.2)
	Weeds	Standard	40.6 (24.4)	7.95 (1.9)
Silage	Annual ryegrass	High	184 (36.1)	401 (43.0)
	Weeds	High	34.9 (13.4)	26.9 (10.3)

Note. Biomass was separated into annual ryegrass and weeds. Nitrogen application level is indicated as high or standard. Values in parenthesis are SE, and $n = 4$ for all means.

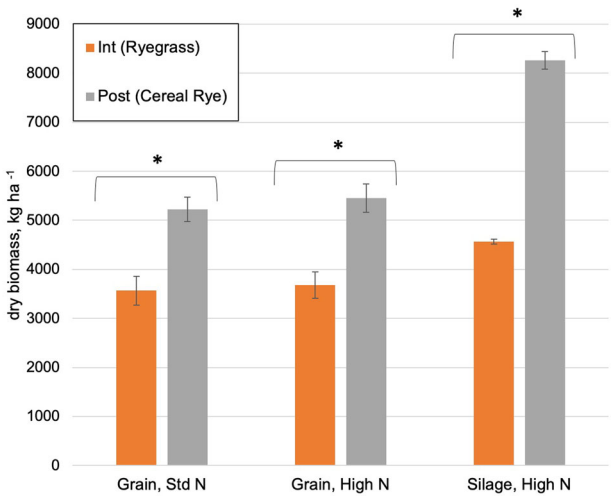


FIGURE 3 Spring aboveground biomass of cover crops (dry biomass, kg ha^{-1}) in grain and silage systems. Corn was interseeded with annual ryegrass (Int, orange bars) or seeded postharvest with cereal rye (Post, grey bars). The grain system received either a standard N (Std N) or high N (High N) application, whereas silage had the high N application only. Error bars represent SE. * represents significant differences at $P < .05$ between cover crop treatments. $N = 4$ for all means

TABLE 2 Corn grain and silage yields (kg ha^{-1})

Corn system	Nitrogen application level	Cover crop	Mean yield
			kg ha^{-1}
Grain	High	Fallow	9,976 (494)
	High	Interseeded	9,953 (1051)
	High	Post	9,912 (371)
	Standard	Fallow	8,868 (652)
	Standard	Interseeded	8,830 (188)
	Standard	Post	7,637 (384)
Silage	High	Fallow	38,719 (1,830)
	High	Interseeded	38,826 (3,849)
	High	Post	35,284 (1,025)

Note. Nitrogen application level is indicated as high or standard; however, at the time of sampling, there had been equal fertilizer applied to all treatments. Fallow, Interseeded, and Post indicate the cover crop treatments of a weedy fallow, interseeded annual ryegrass, and postharvest seeded cereal rye, respectively. Values in parenthesis are SE, and $n = 4$ for all means.

statistically testable hypothesis due to the experimental design as treatments were randomly applied within each cropping system and not across all systems.

In the spring, average percentage of N of the aboveground plant tissue of annual ryegrass ranged from 1.25 to 1.34% N (0.95 confidence interval ± 0.28) and average percentage of N of the aboveground plant tissue of cereal rye ranged from 1.26 to 1.33% N (0.95 confidence interval ± 0.34). In the grain, this translated to about 68 kg N ha^{-1} in cereal rye aboveground biomass and 47 kg N ha^{-1} in annual ryegrass aboveground biomass in both high N and standard N treatments. In the silage, this translated to 109 kg N ha^{-1} in cereal rye aboveground biomass and 57 kg N ha^{-1} in annual ryegrass aboveground biomass.

The CC treatment did not affect the corn silage nor corn grain yields (Table 2), nor did it affect corn ear leaf N content (Supplemental Table S1). The N application level also had no effect on yields as expected because the third application of N in the high N treatments occurred after corn harvest.

3.3 | Microbial respiration and potentially mineralizable N

There were treatment effects on soil respiration from 7-d soil incubations in both grain and silage systems in June samples but not December samples (Figure 4, top panel). In the corn grain system in June, there was a significant effect of N application level on soil respiration ($P = .049$). In the corn silage system, the interseeded treatment had greater C respired than in the fallow treatment ($P = .042$). We observed a general pattern of greater average mg C mg soil^{-1} measured in the fall (December) than in the following spring (June) across all treatments and systems.

Another across-systems pattern was net mineralization of SIN in December and net immobilization of SIN in June

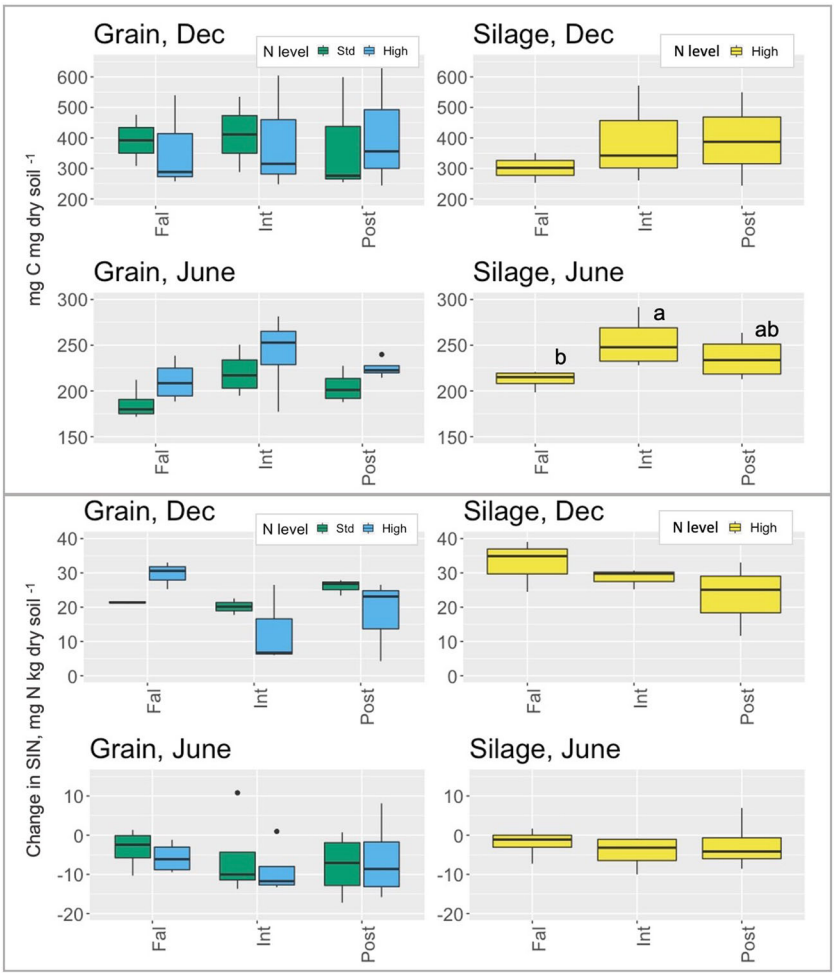


FIGURE 4 Box and whisker plots of CO_2 respiration (mg C mg soil^{-1}) (top panel) and net change in soil inorganic N concentration (mg N kg dry soil^{-1}) (bottom panel) of incubated soils from a field-based cover cropping experiment in corn grain and silage. Green bars represent the standard (Std) N application treatment, and blue bars represent the high N application treatment. Letters "a" and "b" represent Tukey's HSD at $P < .05$. Fal, fallow; Int, interseeded annual ryegrass; Post, postharvest seeded cereal rye

(Figure 4, bottom panel). A positive net change in SIN over the course of this incubation indicated mineralization of N, whereas a negative net change indicated immobilization of N by the microbial community. We did not observe strong treatment effects on the change of total SIN in these incubations.

3.4 | Soil microbial biomass and composition

There were differences in total PLFA biomass in the grain and silage treatments in June but not in December (Figure 5). Both CC treatment and N treatment drove differences in total PLFA biomass in grain. Post hoc pairwise comparisons revealed that the interseeded CC in the high N treatment had significantly greater PLFA biomass than all other treatment combinations (Figure 5).

We found effects of both CC treatment and N application level on the PLFA biomass of individual taxonomic groupings (Table 3). There were no differences in the fungal:bacterial ratio among treatments. The low relative ratios (0.085–0.152) indicate communities dominated by bacteria. There were also no differences in the gram-positive/gram-negative ratios among treatments. These ratios ranged from 0.937 to 1.424. Ratios close to 1 such as these may indicate potential greater diversity than a low value.

Redundancy analyses were performed on grain and silage data using absolute PLFA abundances separated into PLFA biomarker groups (Supplemental Table S2) as well as total PLFA biomass (Figure 6). In the grain system, a permutation test revealed that the model was significant ($P = .001$) and axis 1 of the RDA was significant ($P = .003$). The unconstrained variance was much higher than the constrained variance (64.2 vs. 35.5%), indicating that only a small amount of the variation is displayed in the response matrix. The adjusted R^2 for this model was .283; 34.4% of the variation is explained by RDA1 and 1% is explained by RDA2. These metrics reveal that the results of this analysis should be interpreted with caution as most of the variation was not accounted for. This indicates that variability in this model is driven by untested and unaccounted for factors.

In the silage system, a permutation test revealed that the model was significant ($P = .002$) and that the first axis of this model was significant ($P = .002$) (Figure 6). The adjusted R^2

TABLE 3 Analysis of variance testing effect of cover cropping treatment (CC), N application level (Applied N), and total soil inorganic N (Total SIN) on mean biomass of each phospholipid fatty acid (PLFA) group and total PLFA biomass

System	Season	Gram positive	Actinomycete	AM fungi	Eukaryote	Gram negative	Saprotroph	Total PLFA biomass
Grain	Fall	Applied N*, Total SIN*	Applied N†	Applied N†	NS	Applied N‡	NS	Applied N†
Grain	Spring	Applied N*, CC*	Applied N*, CC*	Applied N*, CC*	NS	Applied N*, CC*	Applied N†, CC*	Applied N*, CC*
Silage	Fall	NS	NS	NS	NS	NS	CC†	NS
Silage	Spring	CC*	CC*	CC†	CC*	CC*	NS	CC*

Note. AM fungi, Arbuscular mycorrhizal fungi; NS, nonsignificant.
*Significant at $P = 0.05$.
†Significant at $P = 0.1$.

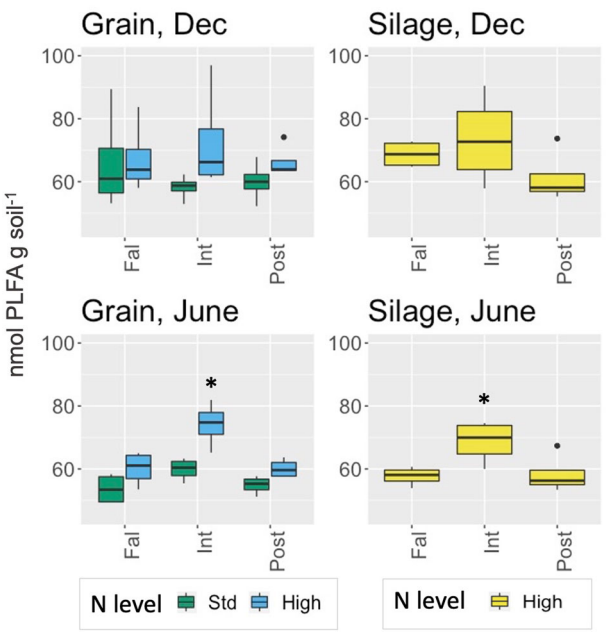


FIGURE 5 Box and whisker plots of total phospholipid fatty acids (PLFA) (nmol PLFA g soil^{-1}) in soils from grain and silage systems with three cover crop treatments. The grain system had a standard N application treatment (green) and a high N application treatment (blue). The silage system had only high N (yellow). * represents a significant difference based on post hoc Tukey HSD pairwise comparisons at $P = .05$. Fal, fallow; Int, interseeded annual ryegrass; Post, postharvest seeded cereal rye

was .473. In this model, the unconstrained variance was lower than the constrained variance (43.6 vs. 54.9%) so the explanatory variables are accounting for more of the variation in the response data; 53.7% of the variation is explained by RDA1 and 1% is explained by RDA2.

In these models, the angles between vectors (or projected vectors onto centroids for qualitative explanatory variables) indicate linear correlation (Figure 6). In the grain system, the RDA triplot shows a positive correlation between total SIN and the abundance of both gram-negative and gram-positive bacteria. Total microbial biomass was positively correlated with both interseeded CCs and the high N application. Again, it is important to note that, due to the low percent of variation explained by these models, these correlations were not strongly supported by this analysis. In the silage system, the triplot showed the same pattern of positive correlations between total SIN and gram-negative and gram-positive groups, as well as a positive correlation between total microbial biomass and interseeded CCs.

Following analysis of the 16S rRNA gene data, a total of 47 phyla were detected among all samples. Across all treatments, seasons, and systems, the phyla that were highest in relative abundance in the soils remained consistent (Acidobacteria, Actinobacteria, Bacteriodota, Chloroflexi, Firmicutes, Gemmatimonadota, Myxococcota, Proteobacteria, and Verrucomicrobiota) (Supplemental Figure S2). Proteobacteria was the most abundant phylum across all treatments in both corn grain and corn silage. There were no differences observed among CC treatments or N application levels at the phylum level. There were also no clear differences in bacterial Bray–Curtis dissimilarity among treatments (Supplemental Figure S1). Finer scale analysis within just the Proteobacteria phylum revealed that Gammaproteobacteria and Alphaproteobacteria were present in all treatments, with Sphingomonadalaes, Rhizobiales, and Burkholderiales orders among the most abundant (Figure 7). In the silage system, sampling date (December vs. June) was a significant predictor variable for Gammaproteobacteria relative abundance ($P = .049$), and the interaction of date and CC treatment was significant for Alphaprotebaceria abundance ($P = .045$) (Figure 7). The date by CC treatment interaction was also a significant predictor of Gammaproteobacteria relative abundance in the grain standard N treatment ($P = .035$). There were no significant differences found among treatments in N-cycling pathways as classified by KEGG pathway maps from the PICRUSt2 software.

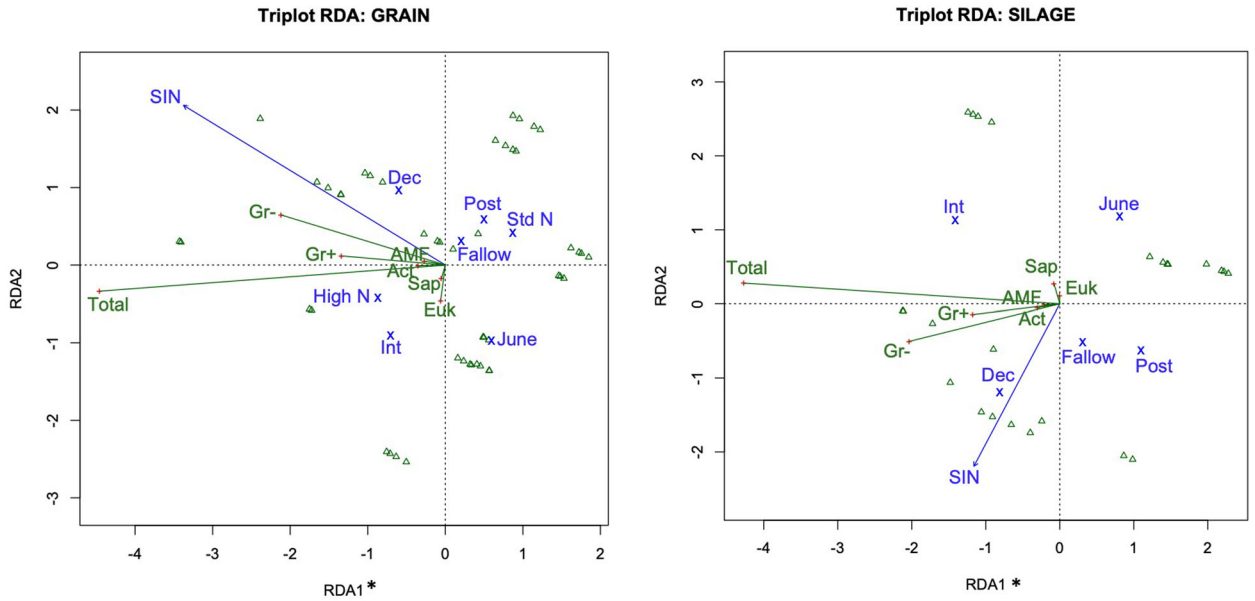


FIGURE 6 Correlation triplots using fitted site scores for two redundancy analyses of soil microbial communities in a corn grain system with two N levels (left) and a corn silage system (right). The angles between variables reflect their correlations. Qualitative explanatory variables are represented as a centroid (blue x). Explanatory variables include sampling date (Dec, December 2017; June, June 2018), applied N level (High N or Std N), soil inorganic N concentration (SIN), and cover crop treatment (Fal, fallow; Int, Interseeded annual ryegrass; Post, postharvest seeded cereal rye). "Species" in this analysis were total phospholipid fatty acid quantities of six phospholipid fatty acid biomarker groups (in green are Act, Actinomycetes; AMF, AM fungi; Euk, Eukaryote; Gr+, gram-positive, Gr-, gram-negative, Sap, saprophytic fungi) and total phospholipid fatty acid biomass (Total). Axes labeled with an asterisk are significant



FIGURE 7 Krona graphs showing soil bacterial relative abundances within the phylum Proteobacteria from a corn silage system in the high N application treatment in June. Bacteria are grouped at the order level. On the left is bacterial abundance from the interseeded annual ryegrass cover crop treatment. On the right is bacterial abundance from the fallow treatment

4 | DISCUSSION

Soil N data indicate that the strategy of interseeding a grass CC may, in certain scenarios, provide equal or greater N retention benefits than a standard practice of postharvest CC planting. In the fall in the silage system, SIN in the top 10 cm was drawn down by the interseeded annual ryegrass (Figure 1). This drawdown of SIN occurred after silage harvest when the interseeded CC had an increase in light availability and could grow at a higher rate during this unshaded period. This pattern of decreased SIN in the fall was not observed in the grain system. We surmise that the interseeded annual ryegrass CC did not have enough time under optimal growing conditions (i.e., unshaded conditions) to take up a significant amount of SIN in the grain system because grain harvest was almost a month after silage harvest. Interseeded annual ryegrass establishes under the corn crop before canopy closure but does not put on substantial biomass until there is light and water available after corn senescence (Brooker et al., 2020) or harvest. Especially in high SIN systems where losses from soil SIN in the fall may be high, this fall growth window is an important consideration when relying on interseeded CCs to target N retention.

In the spring in the high N application treatment, both interseeded and postharvest CC treatments had less SIN at depth than the fallow treatment (Figure 2). This indicates that the interseeded CC decreased N losses via leaching of SIN, as did the postharvest cereal rye CC. This is a noteworthy result as other work has shown cereal rye to outperform annual ryegrass in terms of N scavenging and retention due to inherent differences in species traits between these two grasses (Shipley et al., 1992). In another experiment at the same site, Kaye et al. (2019) directly measured N leaching rates and found that even low concentration differences of SIN at depth were associated with large differences in leaching rates. This work used lysimeters to measure N leaching rates and found that lysimeter N concentrations in cereal rye and CC mixtures containing cereal rye were less than 10 mg N L^{-1}, which was statistically less than in fallow treatments (which at times reached 50 mg N L^{-1}).

Deep SIN from our experiment showed the same pattern as the deep soil SIN from Kaye et al., 2019, with grass CC treatments having significantly reduced SIN in deeper soil layers relative to the fallow treatment, supporting the conclusion that CC treatments reduced N leaching, and that the postharvest cereal rye and the interseeded annual ryegrass had similar capacities to reduce nitrate loss.

In June (the spring after corn harvest), there was more aboveground biomass produced by cereal rye than annual ryegrass in all systems, resulting in more N retained in the cereal rye biomass than in the annual ryegrass biomass (Figure 3). It is important to consider that farmers may terminate their CC earlier than June. For example, if this cropping system was integrated into a corn–soybean rotation in the northeastern United States, these CCs would have been terminated in early May (as in Isbell et al., 2021 and Kaye et al., 2019). By June, the grass CCs presumably had higher C/N ratios than if samples had been collected in early May, influencing spring N dynamics and potential N availability to the subsequent crop (Alonso-Ayuso et al., 2014; Thorup-Kristensen & Dresbøll, 2010). Other research from this field site reports %N content of cereal rye harvested in early May as 1.1–1.4%N and annual ryegrass at 2.15% (data not shown). In this experiment in June, average %N was 1.3% for all grass CCs; there was not a difference in %N between the species nor was the %N affected by N treatment level. If a grower is planning to terminate a CC earlier in the spring, especially under high SIN conditions, an interseeded CC may perform as well as the postharvest cereal rye in terms of spring N retention and provisioning. If terminated later in the spring, the cereal rye will most likely have more N contained in aboveground biomass, and therefore may provide greater N retention and long-term N provisioning services, although in the short-term may tie up soil N during CC decomposition. The C/N ratio of the CC at the time of termination will affect N mineralization rates as the CC decomposes (Finney et al., 2016), which should be considered when planning for the N needs of the following cash crop.

Soil microbial activity and biomass indicated that CC treatment did influence microbial dynamics in ways that affected N cycling in these systems. Interseeded CCs fueled higher microbial respiration, and high N application increased the magnitude of this pattern. Both C and N dynamics were affected by seasonal changes; in the fall, soil respiration data indicated similar levels of microbial activity among all treatments, however in the spring there was higher activity in interseeded treatments (Figure 4). This pattern persisted in total microbial biomass data, as measured by PLFA biomass (Figure 5). Generally, microbial biomass averaged higher in the fall than in the spring across all systems, and in the spring the interseeded high N treatments had higher microbial biomass. Additionally, RDA on PLFAs (Figure 6) demonstrated that microbial biomass played a role in differentiation of treatments. These data support our hypothesis of increased microbial biomass in interseeded systems because of increased C inputs from living roots, leading to increased N retention in this living microbial fraction. However, our expectation of higher spring microbial biomass than in the fall due to higher biomass of CC living roots was not supported.

Even though our data supported our hypothesis of increased microbial biomass from CCs leading to increased N retention, we did not directly measure the potential for the soil microbial pool as a significant source of N. The actual value of N contained in the microbial biomass would need to be within an order of magnitude of other N fluxes in these systems (such as total N uptake by crops or total N losses via leaching)

for this pool to be a significant target of agronomic management. The stoichiometric ratio of microbial C/N/P is relatively conserved in soils (Cleveland & Liptzin, 2007), although N additions, soil properties, and management will affect this ratio with cascading impacts on nutrient cycling (Kallenbach & Grandy, 2011; Liu et al., 2020). A meta-analysis in agricultural systems found that microbial biomass N averaged 44 mg N kg soil^{-1} ± 2.1 (SE) (Kallenbach & Grandy, 2011). Estimating that there are 1.8 million kg of soil in a hectare in the top 15 cm with a bulk density of 1.2 g cm^{-3}, there would be 79.2 kg N ha^{-1} contained in soil microbial biomass. Therefore, soil microbial biomass N has the potential to be a significant N pool in the mass balance of organic corn systems.

We expected to see more pronounced differences in total microbial biomass among CC treatments. One potential reason for this result is the high soil C inputs into these systems from corn residues, which could be obscuring a stronger signal of differences in the microbial biomass driven by CC species or SIN. Additionally, this microbial signal in bulk soil might not be as pronounced after just one season of CCs and might increase after multiple years under this management. Further work in a more controlled environment, sampling at different scales (i.e., rhizosphere soil), or sampling in multiyear interseeded systems might reveal stronger trends or patterns.

There were differences in the composition of fungi and bacteria among treatments as detected with the PLFA analysis. We found a correlation between gram-negative bacteria and SIN (Table 3) and also found that Proteobacteria (a phylum consisting of gram-negative bacteria) was the most relatively abundant phylum represented in these soils (Supplemental Figure S2.) By dividing the Proteobacteria phylum to the gamma and alpha proteobacteria classes, we did find that CCs were significantly driving taxonomic patterns in the silage system. This is further evidence that in the silage system, where CCs were given a longer window for unshaded growth, the CCs may be having more of an effect on microbial dynamics.

These taxonomic data are evidence that the CCs were not only important in taking up and retaining N, but that they affected the potential function of the microbial community as well. Though we were surprised to not find large taxonomic differences among treatments and to not find any differences in predicted metagenomic N cycling functions, this does not necessarily mean that the function of soil bacteria was unchanged; environmental conditions and interactions can alter bacterial function even if the relative abundance of taxa remains similar. Further work to examine the function of soil bacteria, as well as fungi, viruses, and other soil organisms could reveal important information about N cycling and retention in agricultural systems using CCs.

5 | CONCLUSION

Cover cropping has known benefits on C and N cycling in agricultural systems. Interseeding is an innovative and achievable approach to integrate CCs in corn systems to realize these benefits. In terms of N retention services, interseeded grass CCs do have potential to outperform a postharvest seeded CC in the fall under high SIN conditions with a longer window for unshaded fall growth (as in a corn silage system), and may perform just as well as a postharvest seeded CC in the spring. We found no significant effect of CC treatments on corn yield. Interseeded treatments under high SIN conditions had increased microbial biomass with potential for greater N retention in this living microbial fraction. Interseeding CCs is a growing practice among farmers across the United States, and this is some of the first work to assess effects of this practice on N dynamics under different soil N conditions. These results show that the practice of interseeding CCs can be integrated into corn cropping systems to target N retention services, with potential to outperform the standard practice CC strategy.

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AUTHOR CONTRIBUTIONS

Sarah Abigail Isbell: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Visualization; Writing – original draft; Writing – review & editing. María Alonso-Ayuso: Conceptualization; Data curation; Formal analysis; Methodology; Visualization; Writing – review & editing. Terrence H. Bell: Conceptualization; Funding acquisition; Methodology; Resources; Supervision; Validation; Writing – review & editing. Brosi Bradley: Data curation; Investigation; Methodology; Project administration; Validation; Writing – review & editing. Taran Rowles: Formal analysis; Investigation; Methodology; Project administration; Visualization; Writing – review & editing. Jason P Kaye: Conceptualization; Funding acquisition; Investigation; Methodology; Resources; Supervision; Writing – review & editing.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

Abdin, O., Coulman, B. E., Cloutier, D., Faris, M. A., Zhou, X., & Smith, D. L. (1998). Yield and yield components of corn interseeded with cover crops. *Agronomy Journal*, *90*, 63. https://doi.org/10.2134/agronj1998.00021962009000010012x

Alonso-Ayuso, M., Gabriel, J. L., & Quemada, M. (2014). The kill date as a management tool for cover cropping success. *PLOS ONE*, *9*, e109587. https://doi.org/10.1371/journal.pone.0109587

Apprill, A., McNally, S., Parsons, R., & Weber, L. (2015). Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquatic Microbial Ecology*, *75*, 129–137. https://doi.org/10.3354/ame01753

Baributsa, D. N., Foster, E. F., Thelen, K. D., Kravchenko, A. N., Mutch, D. R., & Ngouajio, M. (2008). Corn and cover crop response to corn density in an interseeding system. *Agronomy Journal*, *100*, 981. https://doi.org/10.2134/agronj2007.0110

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using Lme4. *Journal of Statistical Software*, *67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01

Belfry, K. D., & Van Eerd, L. L. (2016). Establishment and impact of cover crops intersown into corn. *Crop Science*, *56*, 1245. https://doi.org/10.2135/cropsci2015.06.0351

Brooker, A. P., Renner, K. A., & Sprague, C. L. (2020). Interseeding cover crops in corn. *Agronomy Journal*, *112*, 139–147. https://doi.org/10.1002/agj2.20046

Buyer, J. S., & Sasser, M. (2012). High throughput phospholipid fatty acid analysis of soils. *Applied Soil Ecology*, *61*, 127–130. https://doi.org/10.1016/j.apsoil.2012.06.005

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*, 581. https://doi.org/10.1038/nmeth.3869

Caswell, K., Wallace, J. M., Curran, W. S., Mirsky, S. B., & Ryan, M. R. (2019). Cover crop species and cultivars for drill-interseeding in mid-Atlantic corn and soybean. *Agronomy Journal*, *111*, 1060–1067. https://doi.org/10.2134/agronj2018.08.0511

Cleveland, C. C., & Liptzin, D. (2007). C:N:P stoichiometry in soil: Is there a "Redfield ratio" for the microbial biomass? *Biogeochemistry*, *85*, 235–252. https://doi.org/10.1007/s10533-007-9132-0

Curran, W. S., Hoover, R. J., Mirsky, S. B., Roth, G. W., Ryan, M. R., Ackroyd, V. J., Wallace, J. M., Dempsey, M. A., & Pelzer, C. J. (2018). Evaluation of cover crops drill interseeded into corn across the mid-Atlantic region. *Agronomy Journal*, *110*, 435–443. https://doi.org/10.2134/agronj2017.07.0395

Curran, W. S., Hoover, R. J., Roth, G. W., Wallace, J. M., Dempsey, M. A., Mirsky, S. B., Ackroyd, V. J., Ryan, M. R., & Pelzer, C. J. (2018). Evaluation of cover crops drill-interseeded into corn across the mid-Atlantic. *Crops & Soils*, *51*, 18.

Dean, J. E., & Weil, R. R. (2009). Brassica cover crops for nitrogen retention in the mid-Atlantic coastal plain. *Journal of Environmental Quality*, *38*, 520–528. https://doi.org/10.2134/jeq2008.0066

Dickey, J. B. R. (1947). Efficient corn growing *(Circular 305)*. Pennsylvania State College Agricultural Extension Service.

Doane, T. A., & Horwáth, W. R. (2003). Spectrophotometric determination of nitrate with a single reagent. *Analytical Letters*, *36*(12), 2713–2722. https://doi.org/10.1081/AL-120024647

Douglas, G. M., Maffei, V. J., Zaneveld, J. R., Yurgel, S. N., Brown, J. R., Taylor, C. M., Huttenhower, C., & Langille, M. G. I. (2020). PICRUSt2 for prediction of metagenome functions. *Nature Biotechnology*, *38*(6), 685–688. https://doi.org/10.1038/s41587-020-0548-6

Finney, D. M., Buyer, J. S., & Kaye, J. P. (2017). Living cover crops have immediate impacts on soil microbial community structure and function. *Journal of Soil and Water Conservation*, *72*, 361–373. https://doi.org/10.2489/jswc.72.4.361

Finney, D. M., Eckert, S. E., & Kaye, J. P. (2015). Drivers of nitrogen dynamics in ecologically based agriculture revealed by long-term, high-frequency field measurements. *Ecological Applications*, *25*, 2210–2227. https://doi.org/10.1890/14-1357.1

Finney, D. M., White, C. M., & Kaye, J. P. (2016). Biomass production and carbon/nitrogen ratio influence ecosystem services from cover crop mixtures. *Agronomy Journal*, *108*(1), 39–52. https://doi.org/10.2134/agronj15.0182

Franzluebbers, A. J., Haney, R. L., Honeycutt, C. W., Schomberg, H. H., & Hons, F. M. (2000). Flush of carbon dioxide following rewetting of dried soil relates to active organic pools. *Soil Science Society of America Journal*, *64*, 613–623. https://doi.org/10.2136/sssaj2000.642613x

Gardner, J. B., & Drinkwater, L. E. (2009). The fate of nitrogen in grain cropping systems: A meta-analysis of 15N field experiments. *Ecological Applications*, *19*(8), 2167–2184. https://doi.org/10.1890/08-1122.1

Grabber, J. H., Jokela, W. E., & Lauer, J. G. (2014). Soil nitrogen and forage yields of corn grown with clover or grass companion crops and manure. *Agronomy Journal*, *106*(3), 952. https://doi.org/10.2134/agronj13.0466

Greene, C., Ferreira, G., Carlson, A., Cooke, B., & Hitaj, C. (2017). Growing organic demand provides high-value opportunities for many types of producers. *Amber Waves*. USDA. https://www.ers.usda.gov/amber-waves/2017/januaryfebruary/growing-organic-demand-provides-high-value-opportunities-for-many-types-of-producers/

Groff, S. (2015). The past, present, and future of the cover crop industry. *Journal of Soil and Water Conservation*, *70*, 130A–133A. https://doi.org/10.2489/jswc.70.6.130A

Howard, M. M., Bell, T. H., & Kao-Kniffin, J. (2017). Soil microbiome transfer method affects microbiome composition, including dominant microorganisms, in a novel environment. *FEMS Microbiology Letters*, *364*, 92. https://doi.org/10.1093/femsle/fnx092

Isbell, S. A., Bradley, B. A., Morris, A. H., Wallace, J. M., & Kaye, J. P. (2021). Nitrogen dynamics in grain cropping systems integrating multiple ecologically based management strategies. *Ecosphere*, *12*, e03380. https://doi.org/10.1002/ecs2.3380

Kallenbach, C., & Grandy, A. S. (2011). Controls over soil microbial biomass responses to carbon amendments in agricultural systems: A meta-analysis. *Agriculture, Ecosystems & Environment*, *144*, 241–252.

Kaye, J., Finney, D., White, C., Bradley, B., Schipanski, M., Alonso-Ayuso, M., Hunter, M., Burgess, M., & Mejia, C. (2019). Managing nitrogen through cover crop species selection in the U.S. mid-Atlantic. *PLOS ONE*, *14*, e0215448. https://doi.org/10.1371/journal.pone.0215448

Ketterings, Q. M., Swink, S. N., Duiker, S. W., Czymmek, K. J., Beegle, D. B., & Cox, W. J. (2015). Integrating cover crops for nitrogen management in corn systems on northeastern U.S. dairies. *Agronomy Journal*, *107*, 1365–1376. https://doi.org/10.2134/agronj14.0385

Liu, X., Lamb, E. G., & Zhang, S. (2020). Nitrogen addition impacts on soil microbial stoichiometry are driven by changes in plant resource stoichiometry not by the composition of main microbial groups in an alpine meadow. *Biology and Fertility of Soils*, *56*, 261–271. https://doi.org/10.1007/s00374-019-01423-1

Matejovic, I. (2003). *The application of Dumas method for the determination of carbon, nitrogen and sulphur in plant samples*. Rostlinna Vyroba - UZPI (Czech Republic). FAO-UN. https://agris.fao.org/agris-search/search.do?recordID=CZ9601053

McLaren, M. R. (2020). *Silva SSU taxonomic training data formatted for DADA2 (Silva version 138)*. https://zenodo.org/record/3731176#.Ymm7Y9rMI2w

McMurdie, P. J., & Holmes, S. (2013). phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLOS ONE*, *8*, e61217. https://doi.org/10.1371/journal.pone.0061217

Nivelle, E., Verzeaux, J., Habbib, H., Kuzyakov, Y., Decocq, G., Roger, D., Lacoux, J., Duclercq, J., Spicher, F., Nava-Saucedo, J.-E., Catterou, M., Dubois, F., & Tetu, T. (2016). Functional response of soil microbial communities to tillage, cover crops and nitrogen fertilization. *Applied Soil Ecology*, *108*, 147–155. https://doi.org/10.1016/j.apsoil.2016.08.004

Noland, R. L., Wells, M. S., Sheaffer, C. C., Baker, J. M., Martinson, K. L., & Coulter, J. A. (2018). Establishment and function of cover crops interseeded into corn. *Crop Science*, *58*, 863–873. https://doi.org/10.2135/cropsci2017.06.0375

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Henry, M., Stevens, H., Szoecs, E., & Maintainer, H. W. (2020). *Package "vegan" Title Community Ecology Package Version 2*. 5–7.

Ondov, B. D., Bergman, N. H., & Phillippy, A. M. (2011). Interactive metagenomic visualization in a Web browser. *BMC Bioinformatics*, 12. https://bmcbioinformatics.biomedcentral.com/articles/10.1186/1471-2105-12-385

Organic Trade Association. (2021). *U.S. organic sales soar to new high of nearly $62 billion in 2020*. https://ota.com/news/press-releases/21755

Parada, A. E., Needham, D. M., & Fuhrman, J. A. (2016). Every base matters: Assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environmental Microbiology*, *18*, 1403–1414. https://doi.org/10.1111/1462-2920.13023

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, F. O. (2013). The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Research*, *41*, D590–D596. https://doi.org/10.1093/nar/gks1219

R Core Team (2018). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. http://www.R-project.org/

Ranells, N. N., & Wagger, M. G. (1996). Nitrogen release from grass and legume cover crop monocultures and bicultures. *Agronomy Journal*, *88*, 777–882. https://doi.org/10.2134/agronj1996.00021962008800050015x

Rankoth, L. M., Udawatta, R. P., Gantzer, C. J., Jose, S., Veum, K., & Dewanto, H. A. (2019). Cover crops on temporal and spatial variations in soil microbial communities by phospholipid fatty acid profiling. *Agronomy Journal*, *111*, 1693–1703. https://doi.org/10.2134/agronj2018.12.0789

Regan, K. H., Voortman, C. A., Wallace, J. M., & Barbercheck, M. E. (2020). Prevalence of early- and late-season pest damage to corn in cover crop-based reduced-tillage organic systems. *Environmental Entomology*, *49*, 865–875. https://doi.org/10.1093/ee/nvaa071

Roth, G. W. (2010). *Organic corn production*. Penn State Extension. https://extension.psu.edu/organic-corn-production

Roth, G. W., Curran, W. S., Wallace, J. M., & Ryan, M. R. (2017). *Cover crop interseeder: Improving the success in corn*. Penn State Extension. https://extension.psu.edu/cover-crop-interseeder-improving-the-success-in-corn

Rusch, H. L., Coulter, J. A., Grossman, J. M., Johnson, G. A., Porter, P. M., & Garcia y Garcia, A. (2020). Towards sustainable maize production in the U.S. upper Midwest with interseeded cover crops. *PLOS ONE*, *15*(4), e0231032. https://doi.org/10.1371/journal.pone.0231032

Schipanski, M. E., Barbercheck, M., Douglas, M. R., Finney, D. M., Haider, K., Kaye, J. P., Kemanian, A. R., Mortensen, D. A., Ryan, M. R., Tooker, J., & White, C. (2014). A framework for evaluating ecosystem services provided by cover crops in agroecosystems. *Agricultural Systems*, *125*, 12–22. https://doi.org/10.1016/j.agsy.2013.11.004

Shipley, P. R., Messinger, J. J., & Decker, A. M. (1992). Conserving residual corn fertilizer nitrogen with winter cover crops. *Agronomy Journal*, *84*, 869–876. https://doi.org/10.2134/agronj1992.00021962008400050020x

Sims, G. K., Ellsworth, T. R., & Mulvaney, R. L. (1995). Microscale determination of inorganic nitrogen in water and soil extracts. *Communications in Soil Science and Plant Analysis*, *26*(1–2), 303–316. https://doi.org/10.1080/00103629509369298

Snapp, S. S., Swinton, S. M., Labarta, R., Mutch, D., Black, J. R., Leep, R., Nyiraneza, J., & O'Neil, K. (2005). Evaluating cover crops for benefits, costs and performance within cropping system niches. *Agronomy Journal*, *97*, 322–332. https://doi.org/10.2134/agronj2005.0322a

Thorup-Kristensen, K., & Dresbøll, D. B. (2010). Incorporation time of nitrogen catch crops influences the N effect for the succeeding crop. *Soil Use and Management*, *26*, 27–35. https://doi.org/10.1111/j.1475-2743.2009.00255.x

de Vries, F. T., & Bardgett, R. D. (2012). Plant–microbial linkages and ecosystem nitrogen retention: Lessons for sustainable agriculture. *Frontiers in Ecology and the Environment*, *10*, 425–432. https://doi.org/10.1890/110162

Wallace, J., Williams, A., Liebert, J., Ackroyd, V., Vann, R., Curran, W., Keene, C., VanGessel, M., Ryan, M., & Mirsky, S. (2017). Cover crop-based, organic rotational no-till corn and soybean production systems in the mid-Atlantic United States. *Agriculture*, *7*, 34. https://doi.org/10.3390/agriculture7040034

Wallace, J. M., Isbell, S., Hoover, R., Barbercheck, M., Kaye, J., & Curran, W. S. (2020). Drill and broadcast establishment methods influence interseeded cover crop performance in organic corn.

Renewable Agriculture and Food Systems, *36*(1), 1–9. https://doi.org/10.1017/S174217052000006X

Wayman, S., Kissing Kucek, L., Mirsky, S. B., Ackroyd, V., Cordeau, S., & Ryan, M. R. (2017). Organic and conventional farmers differ in their perspectives on cover crop use and breeding. *Renewable Agriculture and Food Systems*, *32*(4), 376–385. https://doi.org/10.1017/S1742170516000338

White, C., Barbercheck, M. E., & Curran, W. S. (2015). *Penn State organic crop production guide*. Penn State Extension. https://agris.fao.org/agris-search/search.do?recordID=XF2017001121

Xia, Y., Mitchell, K., Ek, M., Sheffield, J., Cosgrove, B., Wood, E., Luo, L., Alonge, C., Wei, H., Meng, J., Livneh, B., Lettenmaier, D., Koren, V., Duan, Q., Mo, K., Fan, Y., & Mocko, D. (2012). Continental-scale water and energy flux analysis and validation for the North American Land Data Assimilation System project phase 2 (NLDAS-2): 1. Intercomparison and application of model products. *Journal of Geophysical Research: Atmospheres*, *117*(D3), https://doi.org/10.1029/2011JD016048

Youngerman, C. Z., DiTommaso, A., Curran, W. S., Mirsky, S. B., & Ryan, M. R. (2018). Corn density effect on interseeded cover crops, weeds, and grain yield. *Agronomy Journal*, *110*, 2478–2487. https://doi.org/10.2134/agronj2018.01.0010

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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