ABSTRACT

Title of Thesis:	MANAGING WATER, NITROGEN, AND ALLELOPATHY WITH A CEREAL RYE COVER CROP
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A cereal rye (*Secale cereale*) cover crop is a multi-functional tool in a no-till corn agroecosystem. The objectives of this study were to (1) quantify soil phenolic acid concentration under cereal rye shoots and roots, and how tillage impacts their release (2) evaluate the effects of cereal rye termination date on soil water, nitrogen, and corn performance compared to no cover crop. Soil phenolic acids have known allelopathic effects, inhibiting some weed seed germination or growth. Results suggest that cereal rye roots release more phenolic acids into the soil than cereal rye shoots, a novel finding. Results also suggest that corn grain yield following a late-terminated cereal rye cover crop is mediated by precipitation pattern and N release from cereal rye residues. During years of above average summer precipitation a late-terminated cereal rye cover crop does not affect corn grain yields and decreases residual inorganic soil N in the agroecosystem.

MANAGING WATER, NITROGEN, AND ALLELOPATHY WITH A CEREAL
RYE COVER CROP

by

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Introduction

Weed interference accounts for about 50% of crop yield loss in the United States, costing ~$27 billion annually (Soltani et al. 2017). Modern agricultural practices rely on herbicides as the most cost-effective and efficient weed control method (Duke 2012). However, as of 2016, nearly 500 unique cases of herbicide resistant weeds have been identified globally, threatening crop yields worldwide (Heap 2017). Herbicide resistant weeds continue to evolve rapidly in response to the high selection pressure that comes from repeated applications of herbicides with the same mode of action on weeds throughout a crop rotation (Norsworthy et al. 2012). In order to combat further threats from herbicide resistant weeds and reduce the pace of herbicide resistance, growers must implement a multi-tactic weed management approach consisting of multiple best management practices (BMPs) (Norsworthy et al. 2012, Mortensen et al. 2012).

Specific BMPs include the use of primary tillage (i.e. plow, cultimulchers), secondary tillage (mechanical weed cultivation), diversified crop rotation, hand weeding, weed bio-control, and cover crops (Nord et al. 2011). While these approaches can help the combat development and spread of herbicide resistant weeds, some of these approaches also have disadvantages. For example, hand weeding is not economically feasible for large-scale commodity crops, biocontrol efforts can inadvertently expose cash crops to new pathogens or insect pests, and tillage diminishes soil health through reduced soil aggregation and carbon storage, and increased soil erosion. Furthermore, employing any single BMP may provide

significantly less weed control efficacy and consistency than herbicides (Nord et al. 2011). Therefore, there is a need to develop multi-tactic weed management methods utilizing multiple BMP's to diversify selection pressures away from herbicides while increasing the soil health and economic viability of agricultural systems.

Cover crops can play an important role in weed management, as their effect on weeds can work synergistically with herbicides (Teasdale et al. 2005) and diversify overall weed management selection pressure and timing (Nord et al. 2011). For example, within field corn and soybean cropping systems, fall planted cover crops have proven effective at: reducing weed emergence, shifting weed populations to less competitive weeds (Teasdale and Mohler 2000), significantly impacting small-seeded summer annual weeds (Upadhyaya and Blackshaw 2007), and increasing weed seed herbivory (Upadhyaya and Blackshaw 2007). Cereal rye (*Secale cereale* L.) is the most widely used cover crop in the United States (SARE 2014) because it is adapted to a wide range of growing regions, is winter hardy, and produces a large quantity of biomass (Snapp et al. 2005). Cereal rye mulch decomposes slowly, providing a persistent ground cover which has been demonstrated to provide good physical weed control, particularly on small-seeded summer annual weeds (Mirsky et al. 2013), and can suppress weeds through allelopathy (Jabran et al. 2015).

Historically research has focused more on cereal rye physical effects on weed suppression as compared to allelopathic effects (Teasdale and Mohler 1993). Following cereal rye termination in a no-till system the shoot biomass forms a suppressive mulch which physically impedes weed growth, reduces surface light and temperature, and increases soil moisture (Teasdale and Mohler 1993). While it takes

upwards of 8,000 kg ha^{-1} of cereal rye biomass to provide effective management of most summer annual weeds in the US mid-Atlantic region, even 2,500 kg ha^{-1} can reduce weed emergence (Teasdale and Mohler 1993).

While less effective at suppressing weed emergence and survivorship, the release of allelopathic compounds from cover crops can contribute to weed suppression (Singh et al. 2003). Allelopathy is the phytotoxic effect of one plant on another via a chemical pathway (Khanh et al. 2005). It has been shown that allelopathic chemicals may be released from cereal rye shoots or roots through leaching, volatilization, root exudation, or during decomposition of plant residues (Wu et al. 2001). Historically, elucidation of cereal rye allelochemicals has focused on benzoxazinoid compounds while phenolic acids have received less attention (Jabran et al. 2015). Phenolic acids have been shown to chemically inhibit absorption of nutrients and alter cell function via changes to enzyme activity and function, and to weaken oxygen absorption capacity during respiration (Li et al. 2010). Studies have shown that phenolic acids can inhibit germination and reduce growth of weed species including annual ryegrass (*Lolium multiflorum* Lam.), billygoat weed (*Ageratum conyzoides* L.), nut sedge (*Cyperus rotundus* L.), and desert horse purslane (*Trianthema portulacastrum* L.) (Wu et al. 2002, Batish et al. 2009, Khaliq et al. 2010). Phenolic acids can also be released by microbes during microbial degradation of humic substances (Khalid et al. 2002), synthesized by microbes from carbohydrates such as cellulose or starch during plant decomposition (Wojcikwojtkowiak et al. 1990), and formed during decomposition of plant tissue when lignin is catalyzed by the enzyme phenolase in fungi (Ryszkowski et al. 2010).

Few studies characterizing allelopathic effects of phenolic acids from cover crop residues have measured allelochemical release *in situ*, but rather have extrapolated potential allelopathic activity based on tissue concentrations. Blum et al. (1999) called for a shift in allelopathic research focus suggesting that plant-soil interactions in the field are influenced by microbial and abiotic factors. Early field studies on allelopathic effects of cereal rye by Barnes and Putnam (1986) provided evidence that the shoots rather than the roots were the major contributor of allelopathic chemicals into the soil. Studies of cereal rye weed suppression have failed to include comparison managements between shoots and roots which according to Hoffman et al. (1996) is a major omission. Laboratory extracted cereal rye root leachates have been documented to inhibit the growth of tomato (*Solanum lycopersicum* L.), lettuce (*Lactuca sativa* L.) (Barnes and Putnam 1983), barnyard grass and velvet leaf (Hoffman et al. 1996) although the responsible compounds remain undetermined. Carlsen et al. (2008) reported higher overall concentrations of phenolic acids in cereal rye shoot than root tissue; phenolic acid concentration in cereal rye grain has also been measured (Andreasen et al. 2000). None of these studies measured release of phenolic acids into the soil *in situ*.

It is important to measure phenolic acid release under typical management conditions to better understand the allelopathic effect of cereal rye on weed suppression. Therefore, we established a field experiment to determine: (1) the impact of cereal rye shoot and root biomass on the quantity, distribution, persistence, and movement of phenolic acids into the soil during their decomposition, and (2) the

effects of tillage on quantity, distribution, persistence, and movement of phenolic acids from cereal rye residue into the soil.

Methods

Research methods

A one-year field experiment was established at the Beltsville Agricultural Research Center (39.031759N, -76.934591W) to determine the effect of cereal rye shoots and roots on distribution and quantity of phenolic acids in the soil (0-10 cm depth), phenolic acid persistence, and effects of tillage. We implemented a modified split-plot design with four blocks with cereal rye (with and without) as the main plot factor; tillage (with and without) and shoot management (retained or relocated) were the split-plot factors. Thus, a factorial combination of shoots and roots was established in not-tilled plots: +shoots +roots (S/R), +shoots −roots (S/r), -shoots +roots (s/R), -shoots -roots (s/r) (Table 1.1). The tilled management had shoots and roots (S/R tilled), but did not have any managements where shoots or roots were removed (Table 1.1). The experiment was initiated on 24 September 2014 when cereal rye ('Aroostook' cultivar) was planted with a small grain drill at 125.5 kg ha^{-1} on 19 cm row spacing. Plots were 3.1 m by 3.1 m. The no cereal rye plots (s/r) were not seeded and were maintained weed-free with Paraquat (2.2 kg ha^{-1}). The cereal rye was terminated on 23 April 2015 with Paraquat (2.2 kg ha^{-1}) at Zadoks growth stage 53 (Zadoks et al. 1974). The shoot only (S/r) and root only (s/R) plots were established by removing shoots from the s/R plots (cut to the soil surface) and then evenly spreading them onto an area not planted with cereal rye to create the S/r management. Cereal rye shoots were covered with netting to ensure they were not

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blown from S/r plots by wind. In the S/R not-tilled management, the above ground cereal rye biomass was flattened to simulate a roller-crimper (Mirsky et al. 2009). The tilled S/R management was established by tilling shoot and root biomass into the soil with a tractor-mounted rotovator to 10 cm depth. Soils in this region are classified as a Downer-Hammonton complex, which is primarily made up of a loamy sand (~20% or less clay content, ~85% or less sand, ~30% or less silt contents).

Soil samples were collected at 0, 3, 7, 14, 32, and 56 days after termination (DAT) based on expected decomposition kinetics of cereal rye (Poffenbarger et al. 2015) and prior observations that most allelopathic compounds are released within a week after cover crop termination (Rice et al. 2012). On each sampling date, four soil cores were collected randomly from each plot by pushing plastic cylinders (10 cm deep x 4.8 cm in diameter) into the soil to aid in removing intact cores containing undisturbed samples. For the s/R management, two cores were collected directly from within the row and two from between rows to ensure a representative sample. Immediately after collection, soil cores were capped and placed in a cooler with ice packs and transported to the laboratory where they were stored at 4 °C until processed. Cores from each plot were segmented into three depths (0-3 cm, 3-6 cm, and 6-10 cm) and composited by depth at the plot-level. The 0 and 3 DAT soil samples for the S/R tilled cereal rye management were not segmented by depth as the soil was too loose for accurate processing, rather we collected a single bulk sample (0-10 cm). Depth-segmented samples were homogenized by mixing and passing through a 0.6 cm sieve and frozen until analyzed. In the laboratory, soil samples were processed within four hours to reduce microbial activity.

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Shoot and root tissue samples were collected from plants in each block at cereal rye termination. Shoots were cut at the soil surface, cleaned of any soil particles, and frozen for later analysis. Roots were separated from soil, rinsed of soil particles, and frozen for later analysis. Frozen shoot and root tissue samples were freeze-dried to preserve chemical integrity of tissue samples. The samples were then each separately ground using a model 6750 SPEX Freezer/Mill grinder (Metuchen, NJ).

Phenolic acids from cereal rye shoots and roots were extracted separately. To extract phenolic acids from plant tissue samples, we used a modified accelerated solvent extraction method (Carlsen et al. 2008). Clean (oven baked at 400°C) sand was used as the inert support in the extraction cells. Extracts were refined using solid-phase extraction with silica acid columns (Sep-Pak Vac 6cc part # 186004616) from Waters Corp (Milford Mass).

To extract phenolic acids from soil samples, we used a solvent shake and sonication method modified from Macias et al. (2004). A subsample ~2 g of fresh soil was further wetted with 2 mL of water and initially extracted by mixing with 6 mL of methanol. This solvent mix was decanted after centrifugation at for 10 min at 4,000 rotations per minute (rpm) into another vial. Repeated extractions were then performed first with two 20 mL portions of methanol followed by two 20 mL portions of ethyl acetate, each of which were mixed and sonicated for 10 min, and each was centrifuged at 4,000 rpm for 10 min to separate the extraction solvents from the soil solids. All of these solvent mixtures were combined into one extract and concentrated by nitrogen blowdown evaporation and refined using silica acid columns. Cartridges

were pre-cleaned with 5 mL of methanol followed by 5 mL of deionized water and dried by drawing air through the cartridges with suction. Extracts were loaded in 2 mL of solvent and eluted with 5 mL of methanol through the silica acid column. The extracts (~ 7 mL) were concentrated to 3 mL by nitrogen blowdown evaporation.

Phenolic acids in final extracts were separated by liquid chromatography with a C-8 Phenomenex liquid chromatography column (Luna 3 μ-100 Å, 150x4.6 mm, Luna, CA) prior to triple quadrupole mass spectrometry analysis. A solvent gradient was used for delivery into the mass spectrometer. It was operated with a flow of 0.3 ml/min and an initial mixture of three solvents: A) 60:40 methanol: acetonitrile, B) 0.5% acetic acid in water, and C) methanol. For the gradient run on the liquid chromatographer, the initial mix was 3:7 (A:B) which was allowed to gradually change to a 7:3 (A:B) mixture in 10 min, followed by a gradient change from 7:3 (A:B) to a mix of 2:8 (B:C) in 20 min. After this process the column was returned to the initial solvent mixture and equilibrated for the next 9 min in order to run the next sample. The eluted solvents were analyzed using an Ultima-LC Quattro triple quadrupole mass spectrometer (Micromass Ltd., Manchester, UK). Analytes were identified and quantified using negative electrospray introduction and monitored by multiple reaction monitoring using parent to daughter masses [mass defined as mass (m) over charge (z) transitions (m/z)]. The ion transitions that were used are shown in Table 1.2.

Eleven phenolic acids with known allelopathic effects were targeted for detection in the soil and tissue extracts. Of the 11 phenolic acids analyzed, we focused on the four phenolic acids (vanillic acid, 4-hydroxybenzoic acid, ferulic acid,

and coumaric acid) with the greatest concentrations in root tissue and with known allelopathic toxicity (Blum 1999). Extracts were also analyzed for benzoxazinoid compounds, which is reported elsewhere (Rice et al. in review).

Statistical analyses

Differences between shoot and root tissue phenolic acid concentrations at termination were determined with paired t-test analyses ($P < 0.05$). All phenolic acid concentration data were transformed using sqrt(x + 0.5), where x is phenolic acid concentration (Gomez and Gomez 1984) to meet normality assumptions. Data analyses were performed in R (R Core Team 2017). For all analyses on phenolic acids in the soil, DAT was considered a categorical variable. Differences in soil phenolic acid quantity over DAT as impacted by cereal rye shoot management (S/r and s/R not-tilled) were tested via ANOVA (R package *nlme,* Pinheiro et al. 2017). Cereal rye shoot management (shoot vs. root) and DAT were fixed effects, while block was a random effect. Least squares means (R package *lsmeans,* Lenth 2016) were used to compare management and DAT interactions using Tukey's adjusted *P* value for comparing families. The same analytical approach was used to test differences between cereal rye tillage management (S/R not-tilled vs. S/R tilled).

A variance decomposition estimate was calculated to determine which variables significantly impacted data total variance. Four categorical variables and associated interactions were considered when determining estimates of associated variances: DAT, block, management, and depth. The residual unknown variability was also calculated.

Results

We characterized the presence (release dynamics and persistence) of vanillic acid, 4-hydroxybenzoic acid, ferulic acid, and coumaric acid at soil depths of 0-3, 3-6, and 6-10 cm as affected by cereal rye shoot management (shoot vs. root) and cereal rye tillage management (S/R not-tilled vs. S/R tilled). Phenolic acid concentration did not vary by soil depth (data not shown). Variance decomposition analysis indicated that depth accounted for less than 1% of the variability for 4-hydroxybenzoic, coumaric, and ferulic acids and 1.85% for vanillic acid. Therefore, we present our results aggregated across depth.

Cereal rye shoot and root tissue were analyzed separately for phenolic acid concentrations. In general, there were few differences in phenolic acid concentrations between shoot and root tissues (Table 1.3). Of the 11 phenolic acids quantified, vanillic acid, 4-hydroxybenzoic acid, ferulic acid, and coumaric acid were among the five with the highest concentration (Table 1.3). Of the four phenolic acids, coumaric and vanillic acid were present in higher concentrations in root compared to shoot tissue, whereas ferulic acid was present in a higher concentration in shoot compared to root tissue, and 4-hydroxybenzoic acid concentrations did not differ between tissue types (Table 1.3).

In the S/R not-tilled management, soil concentrations of coumaric acid, ferulic acid, and 4-hydroxybenzoic acid varied between cereal rye shoot management (S/r vs. s/R; Table 1.4). Coumaric acid, vanillic acid, and 4-hydroxybenzoic acid concentrations varied over time when pooled by cereal rye shoot management. There were no interactions between cereal rye shoot management and DAT except for ferulic acid in the S/r versus s/R managements. Therefore we present the main effects

only for cereal rye shoot management and DAT for coumaric acid, vanillic acid, and 4-hydroxybenzoic acid (Tables 1.5 and 1.6) while for ferulic acid interaction effects are also presented (Table 1.7).

Soil coumaric acid, vanillic acid, and 4-hydroxybenzoic acid concentrations (pooled across cereal rye shoot management) peaked on 3 DAT (Table 1.6). The rate at which phenolic acid concentrations returned to initial levels was longest for coumaric acid (32 DAT) intermediate for vanillic acid (14 DAT) and shortest for 4-hydroxybenzoic acid (7 DAT; Table 1.6). Ferulic acid concentration remained constant following cereal rye termination in the S/r management (Table 1.7). However, in the s/R management, ferulic acid concentrations at 7 DAT decreased from initial levels but returned to initial levels by day 14 (Table 1.7). Ferulic acid concentration was significantly less in S/r management compared to s/R management, until 3 DAT when the concentration in the S/r management increased to the initial concentration level of the s/R management (Table 1.7).

We also examined the effects of tilled S/R vs. not-tilled S/R on phenolic acid concentrations in the soil (Table 1.8). Tilling S/R reduced 4-hydroxybenzoic acid concentrations compared to not-tilled management, but there was no effect of tillage on coumaric, vanillic, or ferulic acid concentrations (Table 1.9). DAT had an effect on the concentration of all phenolic acids (Table 1.10). Results for vanillic acid including the interaction effect are presented in Table 1.11.

Soil vanillic acid, 4-hydroxybenzoic acid, and coumaric acid concentrations (pooled across tillage management) all increased between 3-7 DAT, but returned to initial concentration levels by the end of the experiment (56 DAT; Table 1.10).

Ferulic acid concentrations decreased over time. Vanillic acid concentrations increased from 0 to 3 DAT in both the tilled S/R and not-tilled S/R managements (Table 1.11). Vanillic acid concentrations were highest on 3 DAT in the tilled S/R management.

Discussion

We observed higher concentrations of vanillic acid and coumaric acid in root compared to shoot tissue (Table 1.3), which is in contrast to Carlsen et al. (2008) who reported overall higher concentrations of phenolic acids in shoot than root tissues. The coumaric acid root concentrations (77.1 µg compound g^{-1} plant dwt) and the ferulic acid shoot concentrations (95.0 µg compound·g^{-1} plant dwt) were consistent with values in Carlsen et al. (2008). Similarly, cereal rye shoot tissue phenolic acid concentrations were similar to the levels reported in Hura et al. (2006). However, cereal rye root phenolic acid concentrations may not be representative of true tissue values due to the possibility that some very fine roots could have been overlooked and not collected.

To date, there is no comprehensive study describing phenolic acid concentrations from cereal rye by soil depth despite the fact that plant root density varies with depth. Nevertheless, our results indicate that phenolic acid concentrations do not vary over the three depth segments of 0-3 cm, 3-6 cm and 6-10 cm. The lack of significant differences by depth is likely due to the root structure of cereal rye and possible soil sorption of phenolic acids. Cereal rye has a fibrous root system with no taproot; roots can extend as far as 230 cm deep (UCANR 2017). Therefore, there would be little difference in root biomass in the top 10 cm to create a soil phenolic

acid concentration gradient in the not-tilled s/R, not-tilled S/R and/or the tilled S/R and not-tilled S/R managements. It has also been shown that coumaric acid, vanillic acid, ferulic acid, and 4-hydroxybenzoic acid all sorb to soil particles, especially to soil clay surfaces and soil organic carbon (Cecchi et al. 2004). The clay content in our study site is not particularly high (20% or less), however, the roots contribute an organic carbon source into the soil, which could enhance phenolic acid sorbtion and thus erase a depth signature (Cecchi et al. 2004). Still, the lack of depth effect in the S/r management is puzzling because in this treatment, one would expect elevated phenolic acid concentrations in the top-most centimeters of soil as shoots rested on the surface. The lack of a depth signature in S/r management suggests that phenolic acids released from shoot tissues were microbially metabolized or rapidly leached to deeper soil depths, rather than being sorbed to soil surfaces (Kuiters and Sarink 1986, Zhang et al. 2010).

We found cereal rye roots were a contributed greater concentrations of phenolic acids to the soil than shoots (Table 1.5). Higher concentrations of coumaric, ferulic, and 4-hydroxybenzoic acids in the soils collected from s/R management compared to S/r (Table 1.5) was likely due to the roots having more contact with the soil than the shoots, which only rested on the soil surface. This conclusion is partially supported by the phenolic acid concentrations in the root biomass, where concentrations of coumaric and vanillic acid were higher than in shoot biomass. However, ferulic acid was present at higher concentrations in the shoot biomass, and there was no difference in 4-hydroxybenzoic acid concentrations. Soil microbial degradation can decrease the concentration of these phenolic acids, addressed below.

Concentrations of coumaric, vanillic, and 4-hydroxybenzoic acid in the cereal rye S/r and s/R managements peaked at 3 DAT and then declined (Table 1.6). The decline back to initial concentration levels could be due to microbial degradation of these compounds (Zhang et al. 2010). It has been documented that 70-99% of the initial quantity of coumaric acid can be degraded by microbial activity within the first 48 hours after release into the soil (Zhang et al. 2010). Blum (1998) found that microbial activity accounted for the majority of the degradation of ferulic acid to vanillic acid after 24 hours; both acids were completely degraded after 150 hours. It has also been documented that the majority of coumaric acid degrades to p-hydroxybenzoic acid within 24 hours, with neither of these phenolic acids present in the soil after 100 hours (Blum 1998). It is possible that higher soil phenolic acid concentrations were observed under the cereal rye s/R management than the S/r management due to increased adsorption of phenolic acids to soil organic matter from the cereal rye roots (Cecchi et al. 2004).

Understanding how tillage affects phenolic acid release from cereal rye and persistence in the soil can be useful in developing new weed management strategies. Our study showed an overall little difference in phenolic acid release from tillage (Table 1.9), which suggests that combining cover crops with no-tillage may work in tandem to physically and chemically suppress weeds. Similarly, Shilling et al. (1985) found that a no-till cereal rye mulch reduced weed biomass by 96, 84, and 83%, respectively, although the allelochemicals and allelochemical concentrations causing this reduction were not identified. However, other studies have shown that tillage

maximizes the allelopathic potential of cover crops (Kruidhof et al. 2014) up to 14 days after tillage.

In this study, all phenolic acids peaked at 3 DAT and then returned to initial concentrations by the end of the experiment in the tilled S/R and not-tilled S/R managements, except ferulic acid (Table 1.10). Ferulic acid decreased from the initial concentration of 7.70 ng·g^{-1} dwt to an ending concentration of 3.08 ng·g^{-1} dwt at 56 DAT for the pooled tilled S/R and not-tilled S/R managements (Table 1.10).

Ferulic acid is transformed to vanillic acid during microbial degradation (Blum 1998). We observe this pattern in the data averaged across tilled S/R and not-tilled S/R managements (Table 1.10). Ferulic acid decreased in concentration from 0 to 7 DAT whereas there was an increase in vanillic acid from 0 to 3 DAT, possibly due to this microbial conversion. Phenolic acids can serve as a carbon source for some microorganisms potentially altering microbial population adapted to metabolism of phenolic acids (Blum 1999). Although this study did not measure microbial respiration or microbial community, all phenolic acids returned to or were below initial soil concentrations by 56 DAT (Tables 1.6 and 1.10), potentially due to microbial degradation of phenolic acids. The decrease in phenolic acid concentrations over DAT (Tables 1.6 and 1.10) could also have been due to abiotic factors such as: the leaching of these water soluble compounds through the soil profile, especially during precipitation events (Batish et al. 2009), or the loss of phenolic acids sorbed to clay surfaces and organic matter

Phenolic acid concentrations measured in this study are three orders of magnitude lower than the potential toxicity thresholds of 100 ppm for coumaric,

vanillic, and ferulic acids (Chou and Patrick 1976). However, cereal rye also releases

other allelopathic compounds, such as benzoxazinoids, which may act synergistically

to inhibit weed growth (Rice et al. 2012, Jia et al. 2006). Phenolic acids can act

additively to reduce weed growth. Ferulic acid and coumaric acid were found to act

additively on perennial ryegrass (*Lolium perenne* L.) and field forget-me-not

(*Myosotis Arvensis* L.) to reduce weed biomass (Jia et al. 2006). Further *in situ*

studies are needed to determine whether the phenolic acids released under the tilled

cereal rye management, vanillic and 4-hydroxybenzoic acid, act additively in tilled

agroecosystems to reduce weed growth (Inderjit and Callaway 2003).

Conclusion

There is a dearth of information on phenolic acid release from cereal rye and

its potential allelopathic effects on weeds. This study examined the primary tissue

source of phenolic acids in cereal rye, the effect of tillage and soil depth on acid

concentrations, and the longevity of acids in the soil. Our study shows of cereal rye

roots contribute phenolic acids to the soil and that tillage and soil depth have a

minimal effect on these acid concentrations. Our research provides direction for

future research specifically towards cereal rye root release of phenolic acids and to

determine factors which contribute to phenolic acid soil concentration loss during

cereal rye decomposition.

Laboratory bioassays, greenhouse assessments, and *in situ* field studies should

be performed to determine factors contributing to cereal rye phenolic acid release,

toxicity of phenolic acids, factors contributing to degradation of acids, and the plant

species the acids negatively affect. Building onto this body of knowledge is necessary

to incorporate allelopathy as part of a multi-tactic weed management approach in

cropping systems.

Table 1.1. Cereal rye tissue treatment names and descriptions.

Treatment name	Treatment description of cereal rye
S/R	Shoots and roots present, not-tilled
S/r	Shoots only on soil surface, not-tilled
s/R	Roots only present, not-tilled
S/R tilled	Shoots and roots tilled

Table 1.2. Phenolic acids parent to daughter transition ions monitored in negative electrospray mode.[1]

Name	Parent>Daughter Ions (m/z)	Retention time (min)
Gallic acid	169.06 > 125.00	7.20
3,4-Dihydroxybenzoic acid	153.10 > 109.14	9.20
Caffeic acid	179.00 > 135.03	11.10
Syringic acid	197.10 > 153.14	11.30
4-Hydroxybenzoic acid	137.13 > 93.20	11.50
Vanillic acid	167.05 > 152.00	11.70
Sinapic acid	222.90 > 164.03	12.90
trans-Coumaric acid[2]	163.10 > 119.10	13.50
cis-Coumaric acid[2]	163.10 > 119.10	14.20
trans-Ferulic acid[3]	193.10 > 134.09	13.60
cis-Ferulic acid[3]	193.10 > 134.09	14.50
2,5-Dihydroxybenzoic acid	153.70 > 109.90	16.10
Salicylic acid	137.37 > 93.04	18.90

[1] Retention time indicates elution of phenolic acids off the C-8 liquid chromatographic column and m/z is the monitored mass (m) divided by its charge (z).
[2,3] Both phenolic acids exist as distinct isomeric pairs, quantities of which were added together to produce total concentrations of coumaric and ferulic acid, respectively.

Table 1.3. Mean cereal rye shoot and root tissue phenolic acid concentration.[1]

Phenolic Acid	Shoots	Roots
	μg compound g^{-1} plant dwt	
Coumaric Acid	31.2 (6.6)	77.1 (19.1)[*]
Vanillic Acid	8.89 (7.8)	42.7 (9.5)[*]
Ferulic Acid	95.0 (34.3)	26.4 (7.1)[*]
Syringic Acid	1.34 (1.1)	12.8 (2.1)[*]
4-Hydroxybenzoic Acid	1.54 (3.19)	5.55 (1.09)
3,4-Dihydroxybenzoic Acid	3.52 (2.10)	4.91 (1.75)
Gallic Acid	2.08 (1.67)	1.17 (0.66)
Caffeic Acid	1.09 (1.19)	1.16 (0.24)
2,5-dihydroxybenzoic Acid	0.25 (0.24)	0.71 (0.41)
Salicylic Acid	0.53 (1.01)	0.52 (0.18)
Sinapic Acid	6.12 (5.89)	0.37 (0.40)

[1] Values are means and standard deviations are in parentheses (n = 3). Asterisks indicate significant difference ($P < 0.05$) according to paired t-tests between plant parts for each phenolic acid.

Table 1.4. Analysis of variance of soil phenolic acid concentration as influenced by cereal rye shoot Mgmt, DAT, and their interaction effect.[1]

Phenolic Acid	Effects	df	F value	$P > F$
Coumaric Acid	Mgmt	1	17.20	0.0001
	DAT	5	3.27	0.01
	Mgmt * DAT	5	1.76	n.s
Vanillic Acid	Mgmt	1	3.44	n.s.
	DAT	5	15.50	<0.0001
	Mgmt * DAT	5	1.10	n.s.
Ferulic Acid	Mgmt	1	7.26	0.01
	DAT	5	1.13	n.s.
	Mgmt * DAT	5	2.40	0.04
4-Hydroxybenzoic Acid	Mgmt	1	17.86	0.0001
	DAT	5	7.75	<0.0001
	Mgmt * DAT	5	0.36	n.s.

[1] Mgmt is cereal rye shoot management (S/r vs. s/R not-tilled) and DAT is cereal rye days after termination.

Table 1.5. Phenolic acid concentration in soil under cereal rye shoot management S/r and s/R not-tilled treatments.[1]

Phenolic Acid	Cereal rye shoot management		P value[3]
	S/r	s/R	
	ng g^{-1} dwt[2]		
Coumaric Acid	14.7 (1.47)	20.0 (2.35)	0.0001
Vanillic Acid	7.57 (0.41)	8.31 (0.65)	n.s.
Ferulic Acid	2.50 (0.23)	3.33 (0.60)	0.0084
4-Hydroxybenzoic Acid	5.43 (0.24)	7.61 (0.60)	0.0001

[1] Values are reported as back-transformed means pooled across sampling time with standard errors in parentheses.

[2] ng g^{-1} dwt is nanograms per gram of dry weight of soil.

[3] P values signify differences between cereal rye shoot management for each phenolic acid.

Table 1.6. Phenolic acid concentration in the soil as a function of DAT in the not-tilled S/r and s/R management combinations.[1]

Phenolic Acid	Days after termination					
	0	3	7	14	32	56
	ng g^{-1} dwt					
Coumaric Acid	10.6 (2.97) b	20.3 (3.29) a	17.5 (2.32) a	17.1 (3.33) a	14.2 (4.07) ab	15.5 (2.10) ab
Vanillic Acid	4.74 (0.25) c	11.5 (1.0) a	10.6 (1.02) ab	6.15 (0.41) bc	6.69 (0.52) c	7.39 (0.6) c
Ferulic Acid	4.08 (1.21)	2.34 (0.26)	2.76 (0.36)	2.54 (0.45)	2.28 (0.16)	2.30 (0.26)
4-Hydroxybenzoic Acid	4.17 (0.22) b	8.0 (0.72) a	6.25 (0.41) ab	5.38 (0.29) ab	7.12 (0.46) a	4.94 (0.80) b

[1] Values are reported as back-transformed means pooled over cereal rye shoot managements and standard errors are in parentheses. Letters represent differences in phenolic acid concentration over time within each phenolic acid. Values are significant at $P < 0.01$.

Table 1.7. Analysis of variance table for ferulic acid interaction between cereal rye shoot management (S/r and s/R) not-tilled and DAT.[1]

Cereal rye shoot management	Days after termination					
	0	3	7	14	32	56
	ng g^{-1} dwt					
S/r	3.03 (1.21) b	2.12 (0.24) b	3.03 (0.42) ab	2.70 (0.52) b	2.07 (0.13) b	2.07 (0.30) b
s/R	7.23 (2.93) a	2.99 (0.72) ab	1.93 (0.60) b	1.60 (0.13) ab	2.94 (0.37) ab	2.94 (0.45) ab

[1] Significant interaction at $P = 0.04$. The values are back-transformed values and standard errors are in parentheses. Letters represent differences in ferulic acid concentration over time and management.

Table 1.8. Analysis of variance of soil phenolic acid concentrations as influenced by cereal rye tillage management and DAT.

Phenolic acid	Effects	df	*F* value	*P* > *F*
Coumaric Acid	Tillage	1	2.33	n.s.
	DAT	5	5.30	0.0003
	Tillage * DAT	5	0.43	n.s.
Vanillic Acid	Tillage	1	3.29	n.s.
	DAT	5	7.69	<0.0001
	Tillage * DAT	5	2.74	0.02
Ferulic Acid	Tillage	1	0.23	n.s.
	DAT	5	5.11	0.0004
	Tillage * DAT	5	0.76	n.s.
4-Hydroxybenzoic Acid	Tillage	1	18.6	<0.0001
	DAT	5	8.61	<0.0001
	Tillage * DAT	5	1.21	n.s.

[1] Tillage management is treatments tilled (S/R) vs. not-tilled (S/R); DAT is days after cereal rye termination.

Table 1.9. Phenolic acid concentration in soil under cereal rye tillage managements.[1]

Phenolic acid	Treatment		
	Tilled S/R	Not-tilled S/R	*P* value
	ng g^{-1} dwt		
Coumaric Acid	11.3 (2.22)	30.5 (3.54)	0.13
Vanillic Acid	11.3 (1.89)	9.09 (0.48)	0.07
Ferulic Acid	3.76 (0.44)	4.34 (0.58)	0.63
4-Hydroxybenzoic Acid	5.19 (1.02)	7.48 (0.44)	<0.01

[1] Values are phenolic acid concentrations pooled over days after termination. The values are back-transformed; standard errors are in parentheses. Cereal rye tillage managements are tilled S/R and not-tilled S/R.

Table 1.10. Phenolic acid concentration in soil as an effect of DAT, pooled over tillage management. [1]

Phenolic acid	Days after termination					
	0	3	7	14	32	56
	ng g^{-1} dwt					
Coumaric Acid	20.8 (5.23) b	45.0 (12.7) a	27.9 (5.12) ab	19.7 (3.95) b	15.1 (3.64) b	16.0 (1.97) b
Vanillic Acid	6.34 (0.63) c	14.1 (2.95) a	11.6 (0.93) ab	9.66 (0.72) bc	8.21 (0.84) bc	8.24 (1.02) c
Ferulic Acid	7.70 (2.01) a	3.98 (0.71) ab	3.85 (0.70) b	3.42 (0.85) b	3.05 (0.42) b	3.08 (0.62) b
4-Hydroxybenzoic Acid	6.02 (0.58) bc	9.87 (1.08) a	10.0 (1.13) a	9.99 (0.74) ab	8.86 (1.29) abc	5.31 (1.01) c

[1] Shown are concentration averages for each day. The values have been back-transformed; standard errors are in parentheses. Letters represent differences in phenolic acid concentration over time within each phenolic acid and are significant at the level of $P < 0.01$. Tillage management is (tilled S/R and not-tilled S/R).

Table 1.11. Analysis of variance table for vanillic acid interaction between tillage management and days after termination.[1]

Tillage management	Days after termination					
	0	3	7	14	32	56
	ng g^{-1} dwt					
Tilled S/R	6.72 (1.36) bc	23.5 (10.5) a	13.6 (2.26) ab	8.57 (0.39) bc	10.1 (1.97) bc	6.50 (0.34) bc
Not-tilled S/R	6.22 (0.73) c	11.0 (1.52) b	11.0 (0.99) bc	10.1 (0.99) bc	7.41 (0.81) bc	8.83 (1.33) bc

[1] Significant at $P = 0.02$. Shown are averages of phenolic acid per each tillage management for each day after termination. The values are back-transformed values and standard errors are in parentheses. Letters represent differences in phenolic acid concentration over time.

Chapter 2: Managing deep inorganic soil N, water, and corn performance from cereal rye

Introduction

Reactive N, such as nitrate, can accumulate in the soil profile when fertilizer nitrogen (N) is applied in excess of corn demand, N availability exceeds corn N uptake, or other resources besides N are limited (Ketterings et al. 2015). Because most soils have low anion holding capacities and nitrate is highly soluble in water, losses from the soil profile can be large during periods of heavy rainfall (Di and Cameron 2002). Leached N comprises 70-90% of N entering the Chesapeake Bay (Pionke et al. 2000), which can lead to eutrophication (Boesch et al. 2001) and hypoxia (Breitburg 2002), presenting significant environmental and economic challenges. In response to environmental concerns, the Maryland Department of Agriculture implemented the Water Quality Cost-Share Program to encourage growers to adopt winter cover crops to maximize periods when plants are utilizing N and thus preventing N loss (MDA 2017).

Cover crops can protect soil from erosion, improve soil organic matter (Reeves 1994), water availability and use efficiency (Sarrantonio and Gallandt 2003), provide pest and disease suppression, increase crop biodiversity (Dabney et al. 2007), and increase nutrient cycling efficiency (Kaspar and Singer 2011). Cereal rye (*Secale cereale* L.) is the most common small grain cover crop in the United States (SARE 2014). It is an excellent N scavenger because it establishes quickly in the fall and produces an extensive deep, fibrous root system (Sarrantonio and Gallandt 2003). Historically, N management with cereal rye has focused on fall N scavenging,

however, there is a considerable amount of time in the spring prior to cash crop planting where a cereal rye cover crop can provide additional N scavenging. The potential to increase N scavenging in the spring and interest in the provision of additional services has led growers to delay cereal rye termination in the spring. For example, delaying management increases cereal rye biomass (Wells et al. 2017), which can promote numerous agroecosystem services such as weed suppression, soil health, and water management. While delaying termination increases biomass and also provides additional N scavenging it increases biomass carbon to nitrogen (C:N), decreases residue quality, of the cover crop as well. Changes in quantity and quality of cover crop biomass have implications for both water and N dynamics and potential consequences for corn growth and development following the cover crop.

Cereal rye decomposition (and concomitant N release) is dependent upon its quality, quantity, and method of termination (i.e. shoot removal, mowing, herbicide, and incorporated) (Reeves 1994, Finney et al. 2016, Krueger et al. 2011, Poffenbarger et al. 2015). Biomass C:N is a good indicator of N mineralization vs. immobilization (Wagger et al. 1998, Nicolardot et al. 2001) with C:N below 25-30 inducing mineralization and C:N above 25-30 triggering immobilization (Jenkinson 1981, Poffenbarger et al. 2015, Janssen 1996). Cereal rye C:N is proportional to growth stage, ranging from ~18-80, and correspond as follows: tillering stage (Zadoks 25) is ~18, the stem elongation to boot stage (Zadoks 30-45) is ~25-40, and anthesis (Zadoks 60) ~50-80 (Alonso-Ayuso et al. 2014, Jenkinson 1981, Plumer 2011). Early-terminated cereal rye (tillering stage) tends to increase soil inorganic N (mineralization), while late-terminated cereal rye (boot-anthesis) tends to decrease

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soil inorganic N (immobilization). In the former case, N losses due to leaching may increase because there are no living plants to utilize mineralized N. However, the latter case may trigger corn N stress. Sufficient soil moisture is crucial for microbial decomposition of plant biomass and N release from the cereal rye to the corn crop (Birch 1958). During dry periods, there is less plant biomass decomposition and N release compared to wet periods (Birch 1958). Therefore, the biomass quality and environmental conditions determine decomposition and N release of the cereal rye.

In addition to N release, cover crop termination timing also influences available soil water content. Growing cover crops affect soil water content primarily through evapotranspiration (Qi and Helmers 2010). When terminated, a cover crop mulch reduces evaporation from the soil surface, when compared to no cover crop (Clark et al. 1997a, Wells et al. 2017, Teasdale 1993) because the soil surface remains covered. Living and terminated cover crop biomass can increase water infiltration by intercepting raindrops, which protects soil particles from detaching and rearranging from soil aggregates (Dabney 1998). This protection prevents soil surface sealing and loss of permeability (Rompkins et al. 1990).

The effects of a cereal rye cover crop, and the termination timing, on water and N dynamics and subsequent corn yields have been evaluated in the mid-Atlantic region (Clark et al. 1997a, 1997b). Previous work shows that delaying cereal rye termination increases soil water availability, has no yield limiting nitrogen effect (at typical fertilizer rates), and increases corn yield. In this previous work, all fertilizer N was applied at sidedress in order to observe early N stress dynamics (Clark et al. 1997a, 1997b) and has focused on N and water dynamics in surface soils (0-20 cm

depth). Mid-Atlantic producers have increasingly used split-N applications (i.e. starter and sidedress) to improve N use efficiency for both optimal yield and environmental stewardship (Khosla and Alley 1999). Therefore, we conducted a study to determine the effects of early- and late-terminated cereal rye on (1) N release from residue, (2) vertical distribution of inorganic soil N, (3) soil water dynamics (0-100 cm), and (4) subsequent effects on corn growth, development, and yield in a no-till under split N applications.

Methods

Research methods

This study was conducted at the United States Department of Agriculture's Beltsville Agricultural Research Center in Beltsville, Maryland (39.03N, -76.90W) within a cropping system experiment, which is part of the National Long-Term Agricultural Research network (Lower Chesapeake Bay-LTAR). The long-term trial, initiated in the fall of 2015, consists of a continuous no-till corn (*Zea mays* L.)-soybean (*Glycine max* (L.) Merr.) rotation with every crop phase present each year. Within both crop phases, there are three cereal rye (*Secale cereale* L.) cover crop treatments (early- and late-terminated cereal rye, and a no cover crop control). Data for this study was collected in the corn phase of the rotation, with corn planted into each cover crop treatment, during 2016 and 2017, and is based on a randomized complete block design with three cover crop treatments and five blocks. Plots were 9.1 m by 9.1 m. The predominant soil type is a Hammonton loamy sand (taxonomic class: Coarse-loamy, siliceous, semiactive, mesic Aquic Hapludults) which are moderately well drained and derived from loamy fluviomarine deposits and on

average about 17% clay, 45% silt, and 38% sand (USDA 2006). The field has an east to west facing slope of 0-2%.

Cereal rye ('Aroostook' variety) was drilled in 7.5 cm rows on 16 October 2015 and 5 November 2016 at 125 kg ha^{-1} using a John Deere 1590 no-till drill. No fertilizer was applied at cereal rye planting. The early-terminated cereal rye was killed on 13 April 2016 (Zadok's 30-31) and 29 March 2017 (Zadok's 29), 43 and 42 days before corn planting, respectively (Zadoks et al. 1974). The late-terminated cereal rye was killed on 20 May 2016 (Zadoks 55) and 29 April 2017 (Zadoks 60), six and 11 days before corn planting, respectively. Early- and late-terminated cereal rye was killed with a combination of 0.6 kg acid equivalent ha^{-1} (kg ae ha^{-1}) of 2,4-D and 0.9 kg ae ha^{-1} of glyphosate in both years.

Corn was planted at a seeding rate of 125 kg ha^{-1} on 26 May 2016 and 10 May 2017 (Pioneer 'P0506AM', DuPont Pioneer®). Genetic traits incorporated into this corn hybrid include: drought tolerance, suitable for reduced tillage, and suitable in corn after soybeans. Corn received 56 kg N ha^{-1} broadcast urea ammonium nitrate (UAN) at planting and a UAN solution was dribbled between rows at sidedress to provide 112 kg N ha^{-1} at growth stage V6 (Hanway 1963) on 26 June 2016 and 20 June 2017.

Cereal rye shoot biomass was collected at early- and late-termination by clipping above ground cereal rye biomass within a 1.0 m^2 quadrat in each plot. Biomass was dried at 60°C for 10 d, weighed, and then ground to pass through a 1 mm mesh sieve. Tissue C and N concentrations were analyzed on 0.2 g subsamples

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using dry oxygen combustion (Leco Corporation, LECO CN628, St. Joseph, MI). Samples were analyzed in duplicate and the values averaged for final C and N concentrations. Additional shoot biomass was collected to measure cereal rye residue decomposition and N release. Fresh biomass was weighed into nylon mesh litterbags (30 cm x 30 cm, 1 mm mesh hole size) to approximate amounts present at early- and late-termination. The bags of residue were placed on the soil surface (six litter bags per block). Litterbags were collected at 0, 4, 12, 15, 20, 24 weeks after early-termination and 0, 4, 8, 11, 15, 20, 24 weeks after late-termination. The decomposed cereal rye biomass from the litterbags was processed as described above. However, to account for soil contamination that occurs while litterbags are in the field, a subsample from each bag was ashed at 400°C to correct litterbag cereal rye weights to an ash-free basis.

To evaluate treatment effects on changes in inorganic soil N in the profile, soil cores to a depth of 100 cm were collected at early-termination (15 April 2016 and 28 and 29 March 2017), late-termination (20 May 2016 and 1 May 2017), just prior to corn side dress (corn growth stage V4 on 14 June 2016 and corn growth stage V5 on 20 June 2017), and after corn harvest (19 October 2016 and 20 October 2017). Four soil cores were collected from each plot (4.6 cm diameter for the first sampling in 2016 and 3.3 cm core diameter for remaining dates) using an AMS Ag-Probe 9100 (AMS, Inc., American Falls, ID). Soil cores were kept in coolers on ice until returned to the laboratory where they were stored at 4°C. The four cores from each plot were sectioned into 0-10 cm, 10-20 cm, 20-30 cm, 30-50 cm, 50-75 cm, and 75-100 cm segments and composited by depth. Soils were air dried, passed through a 2 mm sieve

with rocks collected for correction of bulk density estimates. Inorganic N (NO_3^--N + NH_4^+-N) was extracted using a 1:10 ratio of soil to 1 M KCl by shaking for one hour (Keeney and Nelson 1982). Extracts were filtered and frozen until analyzed for NO_3^--N + NH_4^+-N on a LACHAT QuikChem 8500 series using the cadmium reduction and salicylate protocols, respectively (LACHAT Instruments, Hach Company, Loveland, CO). We report inorganic soil N as the sum of NO_3^--N + NH_4^+-N concentrations, which we converted to kg N ha^{-1} using soil bulk density.

Soil volumetric water content (VWC) was measured at three depths: 0-20 cm, 30-50 cm, and 60-80 cm, 2-3 times per week starting at corn growth stage V5 until corn growth stage R4 in 2016 and R2 in 2017 (9 weeks total in 2016 and 6 weeks total in 2017). Soil VWC was measured with 20 cm trifilar time domain reflectometry (TDR) sensors similar to the Dynamax TR-100 (Dynamax, Inc., Houston, TX). Sensors were installed in the center of each cover crop treatment in all five blocks resulting in 15 instrumented plots. A tractor-mounted post-hole digger was used to excavate to the top of the predetermined depths. Sensors were inserted vertically into soil and holes were refilled and packed to reflect original soil density to eliminate preferential water flow during precipitation events. Soil VWC sensors were installed on 3 July 2016 and 2 July 2017, after corn sidedress fertilizer application, and removed on 7 September 2016 and 14 August 2017.

Sensors were connected manually to a Campbell Scientific TDR100 metallic TDR cable tester and CR10X data logger (Campbell Scientific, Logan, UT). The collected data was converted to soil VWC using TACQ software (Evett 2000). Linear

interpolation was used to estimate soil VWC for days between measurements. We calculated mean soil VWC by treatment for each week for statistical analyses.

Corn performance was assessed by measuring a suite of indicators including biomass, chlorophyll content, leaf area, N content over time, and crop yield. Corn biomass, chlorophyll content, leaf area, and N content were measured at growth stages V5 (23 June 2016 and 16 June 2017) and R2 (1 August 2016 and 26 July 2017) by clipping six representative corn plants at the soil surface from each plot. Chlorophyll content was measured on plants along a 3.1 m section of row in each plot using a SPAD meter (SPAD 502 Chlorophyll Meter, Spectrum Technologies, Inc., Aurora, IL). Measurements were taken at three points on the top collared leaf of each plant and the data were averaged for each plot. Leaf area of photosynthetic leaves was determined by detaching leaves (from harvested plants above) at the collar and measuring the surface area with a LICOR 3100C (LI-COR Biosciences, Lincoln, NE). Leaves that were 50% or more necrotic were not considered photosynthetic and therefore not measured. After SPAD and leaf area measurements were complete, the corn biomass was processed for mass and N content as specified for cover crop biomass.

Statistical analyses

Two sample t-tests were used to determine significant differences in shoot biomass at termination between cereal rye early- and late-terminated treatments, and biomass and N remaining at the end of the corn growing season from the litterbags. Nonlinear regression was used to model cereal rye biomass decomposition and N release based on proportion of initial ash-free weight and N content remaining

in litterbag biomass at each sampling date. A two-part asymptotic exponential decay function (Eq 1) was fit using the R package *nlme* (Pinheiro et al. 2017) to account separately for a rapid and a more resistant biomass fraction (Wieder and Lang 1982) .

$P_t = P_0 * e^{-kt} + (1-P_0)$ Eq. 1

Where P_t is the proportion of biomass or N remaining at a given time t (in degree days, see below), P_0 is the easily decomposable biomass or N fraction, 1- P_0 represents a recalcitrant fraction and k is the exponential decay constant for the easily decomposable fraction (Wieder and Lang 1982). The proportion of P_0 to (1 - P_0) is a characteristic attributed to initial, undecomposed litter. An asymptotic model was used instead of a double exponential decay model because of the short period of this study, the recalcitrant fraction would be mostly resistant to decomposition and the k value (decomposition rate constant) for this fraction would equal 0. This allowed for fitting two parameters instead of three (Table 2.2).

Degree days (DD) were calculated to normalize time based on daily air temperature as follows:

DD= [(T_{MAX} + T_{MIN})/2]-T_{BASE} Eq. 2

Where T_{MAX} and T_{MIN} are daily maximum and minimum air temperature, respectively, T_{BASE} is the base temperature (10 °C) (McMaster and Wilhelm 1997). For days when T_{MIN} or T_{MAX} air temperature was less than T_{BASE}, the T_{MIN} or T_{MAX} was changed to equal T_{BASE}. For days when T_{MAX} was greater than 30 °C, the T_{MAX} was changed to equal 30 °C.

The fitting procedure included block as a random effect and contrasts were used to determine if 'P' or 'k' values were different between early- and late-terminated treatments (paired t-test; R package *lsmeans*, Lenth 2016, Table 2.2). The root mean square error was calculated to evaluate the accuracy of predictions for each model and the coefficient of determination calculated using the 'Cox-Snell pseudo-R^2' value (Cox and Snell 1989) to indicate goodness of fit for non-linear regression.

The law of total variation for regression models is the sum of the variances from multiple independent variables on the dependent variable. By including all observed independent variables in the sum of variances one can observe the unexplained variance due to independent variables not included in the experiment as well as the percent variability from each independent variable on the total variance of the dependent variable (Shedden 2015). Independent variables causing greater than 5% of the dependent variables total variance are considered to impart a significant effect. Therefore, variance decomposition was performed to determine which factors influenced inorganic soil N pools in 2016 and 2017. Factors included in the variance decomposition were: cereal rye termination treatment, soil depth, sampling date, block, and the residual variability. The variance decomposition determined the most significant independent variables causing an effect on the total variance for each sampling date for both years (variability > 5%) were treatment and depth.

A linear mixed-effects (LME) model (R package *lme4*, Bates et al. 2013) was used to determine the effect of cereal rye termination and soil depth on inorganic soil N pools at each sampling date (R core team 2017). We used Box–Cox transformations to satisfy the assumption of homogeneity of variance for the 2016

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post harvest soil samples, and all soil sampling events in 2017 (Box and Cox 1964). Other sampling times in 2016 did not need transformation. Due to depths of variable thickness (i.e. 0-10 cm vs. 75-100 cm), the vertical distribution of inorganic soil N by depth was normalized by 10 cm segments to represent inorganic soil N pools per 10 cm within each depth for statistical analyses. Individual ANOVAs were performed for each soil sampling date with factors determined to have a significant effect on the overall variance from the variance decomposition (Table 2.3); block was included as a random effect in all models. We used Tukey *posthoc* means comparison tests (R package *multcomp*, Hothorn et al. 2017) to determine differences between cover crop treatments and among sampling depths. All values were back-transformed for presentation in the results.

A variance decomposition was used to determine the most significant factors ($P < 0.05$) influencing soil VWC to include in the statistical analyses. A linear model (*lm*) was performed for each year including significant factors and interactions (R package *Stats*, R core team 2017). The research field had obvious visual changes in soil type and slope across the field. Therefore, % clay, % sand, x and y spatial coordinates, and x and y slope coordinates were used as covariates in the model to adjust for spatial differences among VWC collection sites. Each year was analyzed separately. In both years, an ANOVA was used to examine the effect of depth, treatment, and time (weeks between corn growth stages V5 and R4/R2) on VWC. We included significant interactions from the variance decomposition in the models (Table 2.4). Least square means (R package *lsmeans*, Lenth 2016) were used to compare treatment and time effects on soil VWC based on Tukey's adjusted *P* values

for contrasts (Lenth 2016). Least square means representing the mean weekly soil VWC were not back-transformed because they represent means adjusted for the spatial, topographic, and textural variability in the field. Upper and lower 95% confidence intervals (upper and lower CI) were calculated for least square means. All values are considered different at a *P* value of 0.05.

ANOVA was used to test the effect of cover crop and termination timing on corn performance (biomass N content, leaf area, chlorophyll content, population, yield and grain N content). All analyses were analyzed separately for 2016 and 2017. Linear models (R package *nlme*, Pinheiro et al. 2017) were used for the ANOVA (Table 2.5, R core team 2017). Treatment was designated a fixed effect and block a random effect. Contrasts were performed using a Tukey-Kramer post-hoc test (Table 2.6, R package *multcomp*, Hothorn et al. 2017).

Results

Cereal rye shoot biomass, N content, and decomposition

In 2016, cereal rye shoot biomass at termination did not differ between early- and late-termination dates (1.77 and 2.17 Mg ha-1, respectively). However, in 2017, cereal rye shoot biomass was lower for the early- (0.96 Mg ha-1) versus late-terminated treatment (3.25 Mg ha-1; P < 0.0001). The proportion of cereal rye shoot biomass and N content remaining at the end of the corn growing season was lower in both 2016 and 2017 for the early- compared to the late-terminated cereal rye (P < 0.001, P < 0.001).

In 2016 and 2017, the exponential decay model was a good fit for the cereal rye shoot biomass decay and inorganic N content release over time. All but one

model had an R^2 value above 0.90 (Cox-Snell pseudo R^2= 0.97, 0.95, 0.79 and 0.93; Table 2.2; Figures 2.1 and 2.2). The cereal rye biomass decomposition rate and inorganic N release rate (k's) were significantly higher in the early-terminated cereal rye compared to the late-terminated cereal rye in both 2016 and 2017 ($P < 0.0001$ and 0.01, respectively; Table 2.2).

Inorganic soil N-2016

Inorganic soil N dynamics were analyzed separately for each sampling date. Variance decomposition determined that the treatment by depth interaction did not comprise a substantial component of the variance in 2016 inorganic soil N dynamics. There was no difference in inorganic soil N pools (to 1 m) between early- and late-terminated treatments at ~40 days before corn planting, but both cover crop treatments had significantly smaller soil N pools to 1 m (44.6 and 48.1 kg N ha^{-1}, respectively) than the no cover crop treatment (73.4 kg N ha^{-1}; $P < 0.001$). At ~40 days before corn planting, there were larger inorganic soil N pools at 0-10 cm (7.96 kg N ha^{-1}) depth than all other depths. All other inorganic soil N pools did not vary by depth ($P < 0.001$; Figure 2.3).

About 7 days before corn planting, inorganic soil N pools to 1 m depth were similar between the two cover crop treatments (53.0 and 49.8 kg N ha^{-1} , respectively), but significantly smaller than the no cover crop treatment (70.4 kg N ha^{-1}; $P < 0.001$). At this time, soils had the largest inorganic soil N pool at 0-10 cm depth (9.53 kg N ha^{-1}) whereas the 30-50 cm depth (11.1 kg N ha^{-1}), 50-75 cm (11.4 kg N ha^{-1}) and 75-100 cm (11.6 kg N ha^{-1}) had the smallest amount of soil N pool by depth (when normalized for 10 cm depth segments; $P < 0.05$; Figure 2.3).

At the corn side dress (31 days after corn planting), inorganic soil N pools were largest in the no cover crop treatment (68.9 kg N ha^{-1}), intermediate in the early-terminated cereal rye (47.7 kg N ha^{-1}), and smallest in the late-terminated cereal rye (P <0.05; 40.6 kg N ha^{-1}; Figure 2.3). Inorganic soil N pools were significantly larger at 0-10 cm (11.14 kg N ha^{-1}) and the smallest soil inorganic N pools were in the deeper in the soil profile (20-30 cm, 30-50 cm, 50-75 cm, 75-100 cm; $P < 0.05$; Figure 2.3).

At corn harvest, soil inorganic N pools were similar in the cover crop treatments (30.7 and 29.1 kg N ha^{-1}, respectively), but significantly smaller than the no cover crop treatment (52.1 kg N ha^{-1}; $P < 0.001$; Fig 2.3). The largest inorganic soil N pool was in the 0-10 cm depth (11.1 kg N ha^{-1}; $P < 0.05$; Figure 2.3). The smallest inorganic soil N pools were in the deeper soil profile (20-30 cm, 30-50 cm, 50-75 cm, 75-100 cm; $P < 0.05$; Figure 2.3)

Inorganic soil N-2017

At ~40 days before corn planting, inorganic soil N pools to 1 m were largest under the no cover crop treatment (44.0 kg N ha^{-1}) compared to the early- (30.2 kg N ha^{-1}; $P = 0.02$) and late-terminated managements (31.5 kg N ha^{-1}; $P = 0.006$; Figure 2.4). Inorganic soil N pools were larger at 0-10 cm (8.08 kg N ha^{-1}) and 50-75 cm (9.86 kg N ha^{-1}; $P < 0.05$; Figure 2.4) than all other depths. All other inorganic soil N pools did not vary by depth ($P < 0.05$; Figure 2.4).

Inorganic soil N pools to 1 m showed similar patterns among managements at ~7 days. Soil inorganic soil N pools to 1 m were significantly smaller in the late-terminated treatment (10.9 kg N ha^{-1}) than the early-terminated treatment (25.1 kg N

ha^{-1}), and no cover crop treatment (32.8 kg N ha^{-1}; $P < 0.001$, Figure 2.4). At ~7 days

before corn planting, inorganic soil N pools were largest at 0-10 cm depth (8.18 kg N

ha^{-1}) compared to all other depths ($P < 0.01$; Figure 2.4). Due to contamination of

samples with sidedress N fertilizer at corn growth stage V5 those data are not shown.

At post harvest, inorganic soil N pools did not vary between early-terminated

cover crop and no cover crop treatments (103.2 and 89.4 kg N ha^{-1}, respectively) and

were both greater than the late-terminated cover crop treatment (summed inorganic

soil N pool =36.5 kg N ha^{-1}). The largest inorganic soil pool was in 0-10 cm (19.6 kg

N ha^{-1}), 10-20 cm (14.5 kg N ha^{-1}) and 30-50 cm (14.2 kg N ha^{-1}) compared to all

other depths. All other inorganic soil N pools did not vary by depth ($P < 0.05$; Figure

2.4).

Soil volumetric water content
We observed a treatment by depth interaction in 2016. The no cover crop

treatment had lower mean weekly soil VWC in the 0-20 cm soil depth compared to

the 0-20 cm soil depth with a cover crop ($P < 0.001$; Figure 2.5). Further, mean

weekly soil VWC was highest in the late-terminated cereal rye at the 60-80 cm depth

compared to the 60-80 cm depth compared to the other treatments ($P < 0.001$; Figure

2.5). There was a treatment by depth effect in 2017, where mean weekly soil VWC

was highest in the early- and late-terminated treatments at 30-50 cm soil depth

($P<0.05$; Figure 2.6) compared to all other depths by cereal rye treatment

combinations, which did not differ in soil VWC (Figure 2.6).

When examining mean weekly soil VWC by each week in 2016, we observed

that the first week, July 5-11, had the highest mean weekly soil VWC ($P < 0.001$;

Figure 2.7). Between July 5-11, the plots received 39 mm of precipitation,

contributing to the greatest mean weekly soil VWC. The sixth week, August 8-11,

had the lowest mean weekly soil VWC measured across the growing season ($P <$

0.001; Figure 2.7). Although August 8-11 received a total of 65.0 mm of

precipitation, the week prior only received 0.25 mm precipitation, which likely led to

low mean weekly soil VWC.

In 2017, we observed the highest mean weekly soil VWC in weeks 4-6 (July

24- Aug 17; $P < 0.001$) during which time plots received 188.3 mm of precipitation

total. The second week (July 10-16) was the driest period overall ($P < 0.001$). July

10-16 received a cumulative precipitation of 6.4 mm during this week.

Corn performance

To assess the effects of water and N stress, we measured a suite of corn

performance criteria including corn biomass N content, corn leaf area, corn grain N

content, yield, and population. Based on statistical analyses for all corn performance

criteria in 2016, we only observed a significant effect of cover crop treatment on corn

N content at corn maturity and corn grain yield (Tables 2.5 and 2.6). At corn

maturity, biomass N was less in the cereal rye early- and late-terminated (156.0 and

131.0 kg N ha^{-1}) compared to the no cover crop treatment (192.9 kg N ha^{-1}; Tables

2.5 and 2.6). There was no difference in corn grain yields between the no cover crop

(13.3 Mg ha^{-1}) and early-terminated cereal rye (13.4 Mg ha^{-1}; Tables 2.5 and 2.6) in

2016, however, both yielded higher than the late-terminated cereal rye (12.3 Mg ha^{-1};

Tables 2.5 and 2.6; $P < 0.05$).

In 2017, other than corn N content and leaf area at corn growth stage R2, there was no effect from the no cover crop, early- and late-terminated cereal rye on all corn performance criteria including yield (Tables 2.5 and 2.6). At corn growth stage R2, corn biomass N content in the late-cereal rye terminated cereal rye (191.6 kg N ha^{-1}) was higher than the early-terminated cereal rye and no cover crop treatments (148.5 and 164.8 kg N ha^{-1} respectively; $P < 0.001$; Tables 2.5 and 2.6). Similarly, leaf area was larger at corn growth stage R2 in the late-terminated cereal rye (3.69 cm^2) compared to the no cover crop management and early-terminated cereal rye (3.12 and 2.94 cm^2; $P < 0.01$; Tables 2.5 and 2.6). However, the two parameters did not influence corn grain yield.

Discussion

Factors influencing cereal rye performance, decomposition, and N release

Although an increase in biomass accumulation from the early- to late-terminated cereal rye was expected, the lack of difference in 2016 between the early- and late-terminated cereal rye is likely because rainfall in March and April was about half the 30-year average (Table 2.1). The droughty conditions in the early spring resulted in similar growth between termination timings. Furthermore, the variability in soil texture (and thus moisture dynamics) among blocks likely prevented us from detecting and effect of cover crop termination timings on biomass accumulation.

We consistently observed higher rates of biomass decomposition and N release from the early-terminated cereal rye (Table 2.2) compared to late-terminated cereal rye. Differences in decay rates and N release is likely related to the lower C:N ratio of the cereal rye shoot biomass in the early compared to the late-terminated

cereal rye (27:1 and 48:1 in 2016 and 15:1 and 25:1 in 2017). Lower C:N ratios result in more rapid decomposition and decay rates (k), a well-documented pattern (Nicolardot et al. 2001, Alonso-Ayuso et al. 2014, Poffenbarger et al. 2015, Wagger 1998). In 2016, the early-terminated cereal rye released 50% of initial N about one month after termination (Figure 2.2). Our biomass decomposition rates for the late-terminated cereal rye ($k = 0.002$ in 2016 and 0.003 in 2017) were similar to Poffenbarger et al. (2015, $k = 0.0043$), which had a similar termination date as our study.

In 2016, the cereal rye N increased in the late-terminated cereal rye biomass between 20 May 2016 to 10 June 2016 (413 cumulative degree days; Figure 2.2), which is the result of soil N translocation to cereal rye biomass by microbes and fungi, resulting in N immobilization. This increase in cereal rye N could also have been due to potential contamination from corn starter fertilizer

Factors influencing inorganic soil N

Due to cereal rye inorganic soil N uptake for growth and development over the winter and early spring, inorganic soil N pools (to 1 m) at ~40 days before corn planting were depleted in both years under cereal rye treatments when compared to the no cover crop treatment (40% and 30% reduction in 2016 and 2017, respectively), this is consistent with the 35% soil N pool depletion observed by Krueger et al. (2011).

When cereal rye was allowed to grow until ~7 days before corn planting, we observed no difference in soil N depletion under cereal rye treatments when compared to the no cover crop treatment in 2016. We expected that the additional

month of growth in the late-terminated cereal rye would increase biomass and further deplete soil N pools compared to the early-terminated cereal rye, but this was not the case in 2016. This is likely due to the precipitation pattern in 2016. Rainfall levels in March and April were about half the 30-year average; the droughty period stunted cereal rye growth and development and reduced inorganic soil N uptake by the cereal rye (Table 2.1). However, in 2017 at ~7 days before corn planting the late-terminated cereal rye became a sink of soil N during the early stages of decomposition and less N was released during a period when there was no corn to use it; causing early-terminated cereal rye and no cover crop treatments to have larger inorganic soil N pools (to 1 m).

We expect that the differences in precipitation patterns between 2016 and 2017 caused the differences in post-harvest inorganic soil N pools. During the 2016 corn growing season, precipitation levels were similar to 30-year average. However, in 2017, the precipitation patterns were variable over the growing season. During June 2017, the corn received about half as much precipitation as the 30-year average in June (Table 2.1) whereas in July and August the corn received about twice as much as the 30-year average. In 2017, the decrease in soil N availability at the end of the corn growing season in the late-terminated treatment suggests this treatment improved N management because there was no loss of corn yield and little indication of corn N stress based on corn biomass N compared to the other treatments in 2017 (Table 2.6), therefore the late-terminated cereal rye may be facilitating corn N uptake and thus causing N depletion in the soil profile.

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Factors influencing soil N by depth

We suspect that at ~40 days before corn planting there is the greatest amount of inorganic soil N available at the 0-10 cm depth under the early-terminated cereal rye because of soil organic N mineralization in the topsoils as well as cereal rye root development and inorganic N uptake in the deeper soil layers (Krueger et al. 2011, Alonso-Ayuso et al. 2014).

At ~7 days before corn planting there is more inorganic soil N in the surface depth (0-10 cm) than nearly all other depths in both 2016 and 2017 likely due to N mineralization of cereal rye residue (Dabney et al. 2007). The increase in topsoil inorganic N availability is beneficial for early corn development after planting (Kranz et al. 2008).

There is less inorganic N available in the lower soil depths (>30 cm) at corn growth stage V5 and post harvest soil sampling in 2016 and post harvest soil sampling in 2017 likely due to inorganic N uptake by the corn roots *(*Clark et al. 1997a). This reduces susceptible N to leaching loses deeper in the soil profile.

Factors influencing soil volumetric water content

Results from an average precipitation year (2016) indicate that no cover crop leaves the surface soil (0-20 cm) more susceptible to evaporation (Table 2.4; Clark et al. 1997b, Wells et al. 2017, Dabney 1998). Having less surface soil water available for the corn crop could be potentially detrimental for maintaining corn yields under drought conditions. The greatest soil VWC under the late-terminated cereal rye at 60-80 cm is likely due to the cereal rye enhancing soil water infiltration. This water is too deep for the majority of corn root uptake and thus leaves this water susceptible to leaching under an average precipitation year (2016). Under variable precipitation

patterns (2017), there was more soil VWC available under the cereal rye treatments from 30-50 cm. This may be due to enhanced soil water infiltration under the cover crop treatments (Dabney 1998), leaving more soil VWC available for corn root uptake.

Factors influencing corn performance

In 2016, corn biomass N at maturity was higher in the no cover crop compared to the cover crop treatments, which corresponded to lower inorganic soil N available in the cover crop treatments. In 2016, we observed significantly lower corn grain yields in the late-terminated cereal rye compared to the other treatments, which is most likely the result of early season inorganic soil N uptake by the cereal rye and slower release of cereal rye N to the corn crop (Crandall et al. 2005).

In 2017, we observed higher corn biomass N content and leaf area at corn growth stage R2 in the late-terminated cereal rye. This is likely due to the late-terminated cereal rye facilitating N uptake and growth, perhaps by reducing soil water evaporation and increasing soil water infiltration. However, this did not have any consequences for corn grain yield between cover crop treatments, (Tables 2.5 and 2.6), which we believe is due to adequate precipitation and N release from the cereal rye during the corn growing season.

Conclusion

Our work highlights the complex interactions a cereal rye cover crop, and its termination timing, have on nitrogen and water dynamics and subsequent corn performance. In general, a cereal rye cover crop tightens nutrient cycling and

increases soil water availability. However, the timing of its management greatly influenced these effects. Delaying cereal rye termination increases overall N scavenging and typically results in greater biomass quantity. However, regardless of the degree of increase in biomass quantity, the delay in termination reduces the overall quality of the residue (higher C:N). The residue quality plays a larger role in driving the rate and quantity of cereal rye decomposition as compared to quantity. Therefore, delaying termination of cereal rye will decrease both the decay rate and overall decomposed material.

Cereal rye, in general, reduced overall soil inorganic N losses, however higher quantities of a lower quality cereal rye cover crop can have a bigger effect on soil N cycling. These N dynamics are tightly linked to the timing of fertilizer applications, soil water dynamics, and annual precipitation. A cereal rye cover crop tended to increase water infiltration and storage as compared to the no cover crop control. However, delaying cereal rye termination can further increase soil water in the profile; both effects have implications for water provisioning later in the season. The effect of a late-terminated cover crop on corn yield appears to be mediated by precipitation patterns, which control N release from decomposing residues. The Northeastern US is expected to experience higher precipitation (during the spring and summer growing season) due to climate change (IPCC 2014), therefore, we expect that planting cereal rye cover crops will provide multiple agroecosystem benefits (e.g. N retention and water provisioning), while maintaining high corn yields. Further work is necessary to determine how soil fertility management may be adjusted if cover crop termination is delayed.

Table 2.1. Mean ambient temperature and precipitation for the spring and summer of 2016, 2017, and the 30-year average in Beltsville, MD.

Month	Mean ambient temp (°C)			Precipitation (mm)		
	2016	2017	30-year avg.	2016	2017	30-year avg.
March	10	6	7	48	82	93
April	12	16	12	46	107	85
May	16	17	17	149	156	110
June	23	23	23	110	28	94
July	26	25	25	133	209	100
August	25	22	24	120	170	83
September	22	20	20	88	42	104
October	15	16	14	28	93	93

Table 2.2. Parameter estimates of the exponential decay of cereal rye shoot biomass and N content over time. Estimates are accompanied by standard error in parentheses. Letters represent differences between termination timing for each measurement and coefficient ($P < 0.05$).[1]

Year	Measurement	Termination Timing	P†	k	$P > F$	RMSE	Cox-Snell Pseudo R^2
2016	Shoot biomass	Early	0.81 (0.01) a	0.010 (0.01) a			
		Late	0.68 (0.03) b	0.002 (0.000) b	< 0.0001	0.05	0.97
	N content	Early	0.72 (0.04) a	0.004 (0.001) a			
		Late	0.54 (0.21) b	0.001 (0.001) b	0.01	0.14	0.79
2017	Shoot biomass	Early	0.90 (0.02) a	0.005 (0.001) a			
		Late	0.59 (0.02) b	0.003 (0.001) b	< 0.0001	0.07	0.95
	N content	Early	0.94 (0.02) a	0.005 (0.001) a			
		Late	0.77 (0.03) b	0.003 (0.001) b	0.006	0.09	0.93

†P, proportion of cover crop biomass lost after decay; and k, decay rate of cover crop biomass and N loss.
[1] Root mean square error (RMSE) determines how much error there is between the observed values to the modeled values for each model; the closer the value is to 0 the less error. The Cox-Snell Pseudo R^2 is a coefficient of determination used in non-linear models.

Table 2.3. Analysis of variance table for 2016 inorganic soil N at each sampling date as a function of cover crop management (no cover crop, early- and late-terminated cereal rye) and soil sampling depth.

Soil sampling	Effects	2016		2017	
		df	F value	df	F value
Early-terminated cereal rye	Treatment	2	40.3	2	5.74
	Depth	5	42.4	5	7.36
Late-terminated cereal rye	Treatment	2	24.6	2	27.7
	Depth	5	17.1	5	8.26
Corn (V4)	Treatment	2	56.1	N/A	N/A
	Depth	5	25.3	N/A	N/A
Corn harvest	Treatment	2	14.2	2	43.7
	Depth	5	11.4	5	6.31

Table 2.4. Analysis of variance table for 2016 and 2017 soil volumetric water content as influenced by variables.

Effect	2016			2017		
	df	*F* value	*P* > *F*	df	*F* value	*P* > *F*
Treatment	2	4.9	<0.001	2	3.5	<0.05
Depth	2	318.4	<0.01	2	30.3	<0.001
Week	8	52.0	<0.001	5	52.9	<0.001
Clay concentration	1	207.5	<0.001	1	170.3	<0.001
Sand concentration	1	62.1	<0.001	NA	NA	NA
X spatial coordinate	1	4.9	<0.05	1	2.5	0.12
Y spatial coordinate	1	26.0	<0.001	1	54.9	<0.001
XY spatial coordinate	NA	NA	NA	1	22.6	<0.001
X spatial coordinate for slope	1	71.8	<0.001	NA	NA	NA
Y spatial coordinate for slope	1	1.1	0.29	1	1.2	0.27
Depth:Treatment	4	11.1	<0.001	4	3.2	<0.05
Depth:Week	16	7.3	<0.001	NA	NA	NA
Depth:Y spatial coordinate	2	51.7	<0.001	2	19.3	<0.001
Depth:Y spatial coordinate for slope	2	29.3	<0.001	NA	NA	NA

Table 2.5. Analysis of variance table on the effects of no cover crop, early- and late-terminated cereal rye on corn performance criteria (grain yield, biomass and grain N content, population density, leaf area, and chlorophyll content) in 2016 and 2017.

Corn performance	2016			2017		
	df	*F* value	*P* value	df	*F* value	*P* value
Yield	2	1.19	0.04	2	1.19	0.35
Grain N content	2	2.82	0.12	2	0.38	0.70
Corn population	2	0.65	0.55	2	1.22	0.35
Biomass N content (V5)	2	0.17	0.85	2	2.13	0.18
Biomass N content (R2)	2	0.01	0.99	2	6.87	0.02
Biomass N content (BL)	2	10.2	0.01	2	0.74	0.51
Leaf area (V6)	2	1.06	0.39	2	2.47	0.15
Leaf area (R2)	2	0.48	0.64	2	9.86	0.01
SPAD (V5)	2	1.81	0.23	2	1.34	0.32
SPAD (R2)	2	0.21	0.81	NA	NA	NA

Table 2.6. Corn performance for 2016 and 2017 as an effect of cover crop management. Different letters within each corn performance and year represent statistical differences by cover crop treatment ($P < 0.05$)[1].

Corn performance	Cover crop treatment	2016 Means	2017 Means
Grain yield (Mg ha^{-1})	No cover crop	13.3 (0.3) a	10.3 (0.6) a
	Early	13.4 (0.2) a	11.0 (0.3) a
	Late	12.3 (0.6) b	11.1 (0.6) a
Corn Population (plants ha^{-1} x 10^3)	No cover crop	69750 (2109) a	60278 (2723) a
	Early	67167 (2583) a	58556 (2583) a
	Late	68028 (2856) a	63722 (3444) a
Biomass N content (V5) (kg N ha^{-1})	No cover crop	20.7 (3.13) a	7.69 (1.67) a
	Early	22.5 (2.46) a	9.62 (1.40) a
	Late	22.5 (1.79) a	13.8 (3.99) a
Biomass N content (R2) (kg N ha^{-1})	No cover crop	217 (11.3) a	164.8 (9.5) ab
	Early	217 (12.1) a	148.5 (7.7) a
	Late	216 (14.0) a	191.6 (11) b
Biomass N content (BL) (kg N ha^{-1})	No cover crop	192.9 (17.6) a	58.9 (6.46) a
	Early	156.0 (22.0) b	53.7 (2.87) a
	Late	131.0 (35.1) b	60.0 (5.31) a
V6 Leaf area (m^2)	No cover crop	0.50 (0.06) a	0.26 (0.03) a
	Early	0.60 (0.05) a	0.31 (0.03) a
	Late	0.55 (0.03) a	0.38 (0.07) a
R2 Leaf area (m^2)	No cover crop	3.74 (0.13) a	3.12 (0.19) a
	Early	3.85 (0.10) a	2.94 (0.11) a
	Late	3.89 (0.19) a	3.69 (0.12) b
SPAD (V6)	No cover crop	52.5 (1.27) a	49.6 (1.18) a
	Early	54.6 (1.14) a	51.9 (0.73) a
	Late	52.8 (0.70) a	52.6 (1.84) a
SPAD (R2)	No cover crop	59.9 (0.82) a	NA
	Early	60.0 (0.31) a	NA
	Late	60.4 (0.44) a	NA

[1] Shown are averages per each treatment with the standard error in parentheses. Contrasts were performed using Tukey-Kramer post-hoc tests, tested per each corn performance.

Figure 2.1. Modeled 2016 and 2017 proportion of initial mass remaining in litterbags. The lines represent the modeled values for the cereal rye management, whereas the points represent mean observed values.
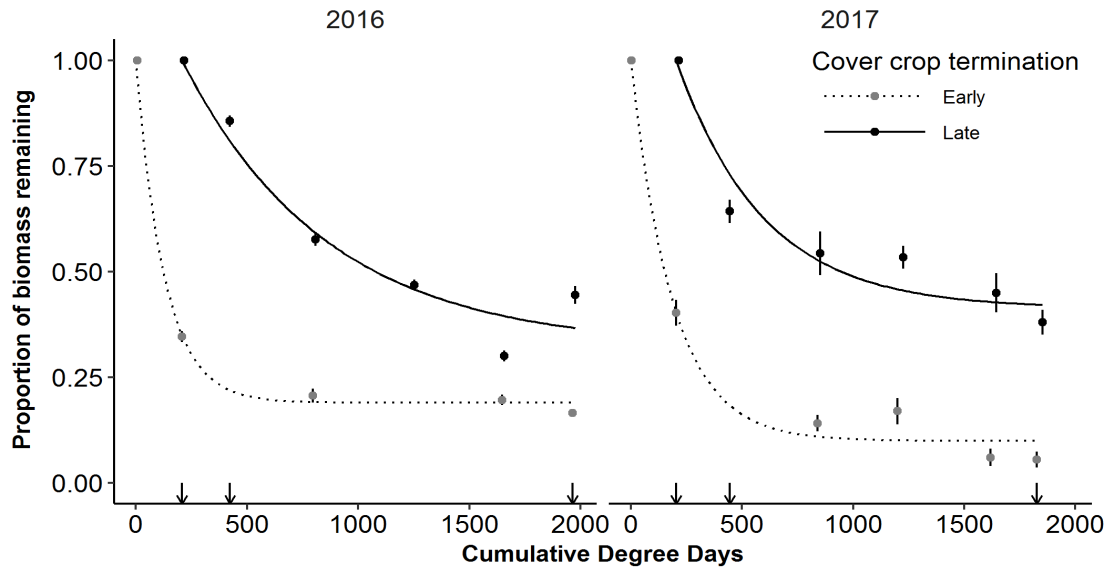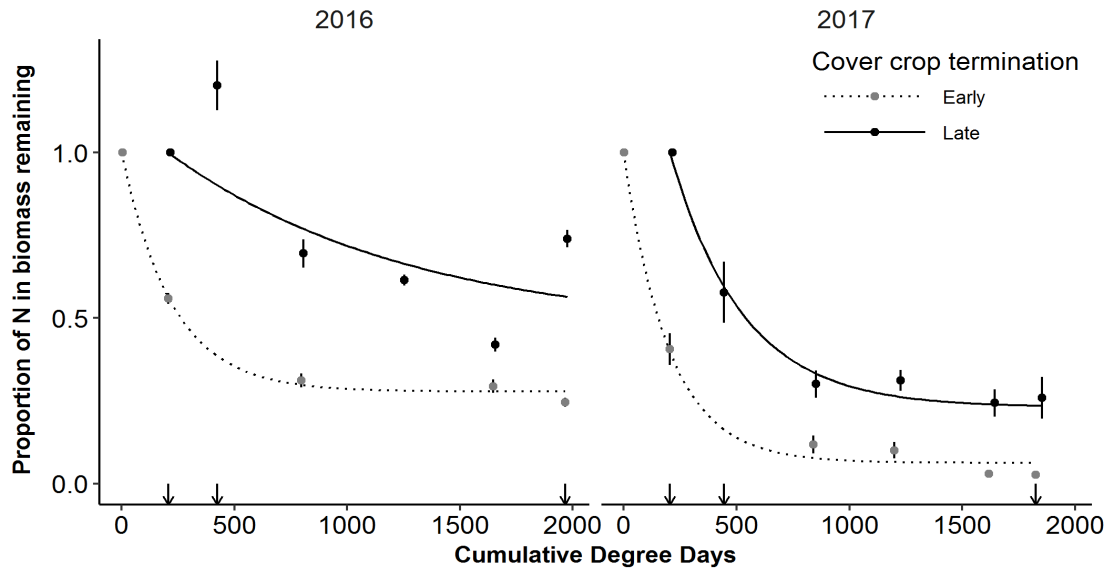
Figure 2.2. Modeled 2016 and 2017 proportion of initial N remaining in litterbags. The lines represent the modeled values for the cereal rye management, whereas the points represent mean observed values.

Figure 2.3. Inorganic soil N at four points during the 2016 corn growing season for no cover crop soil and early- and late-terminated cereal rye management.
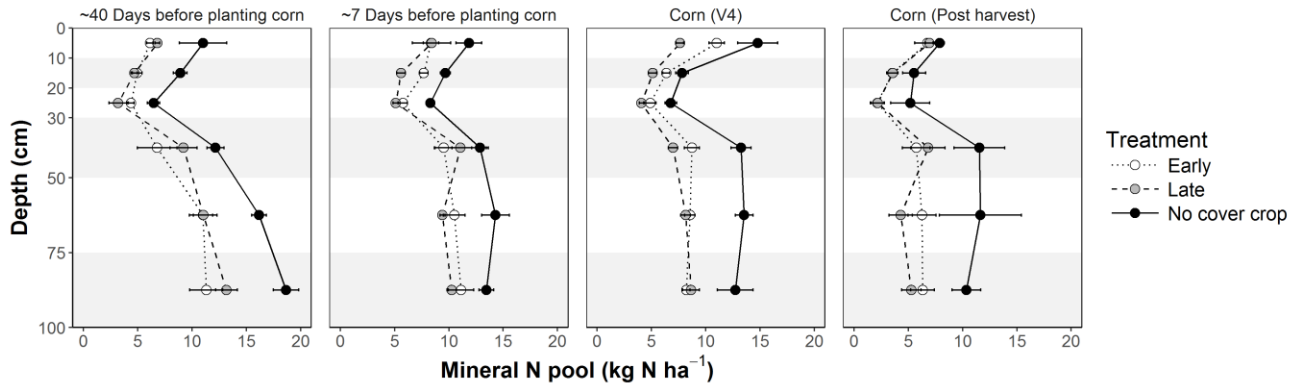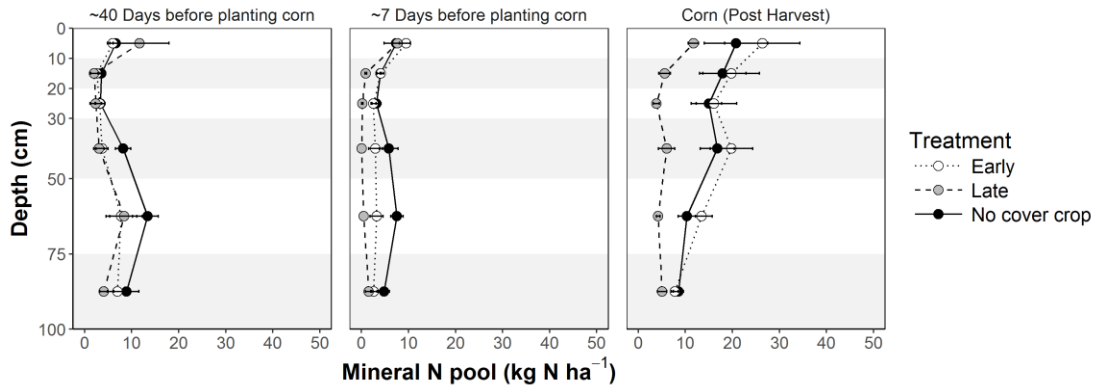
Figure 2.4. Inorganic soil N at three points during the 2017 corn growing season for no cover crop soil and early- and late-terminated cereal rye management.

Figure 2.5. 2016 weekly average soil volumetric water content by depth over treatments.
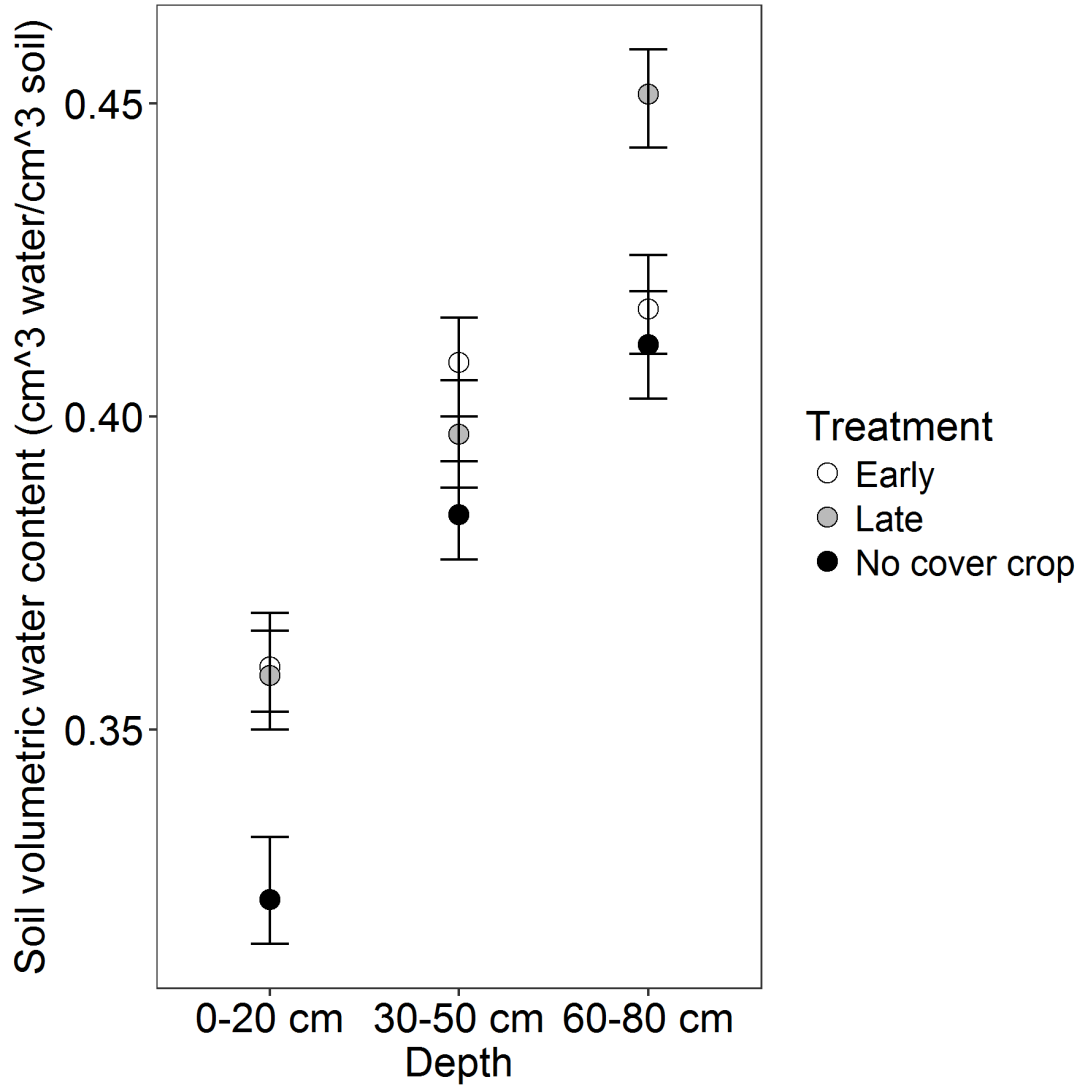
Figure 2.6. 2017 weekly average soil volumetric water content over treatments by depth.
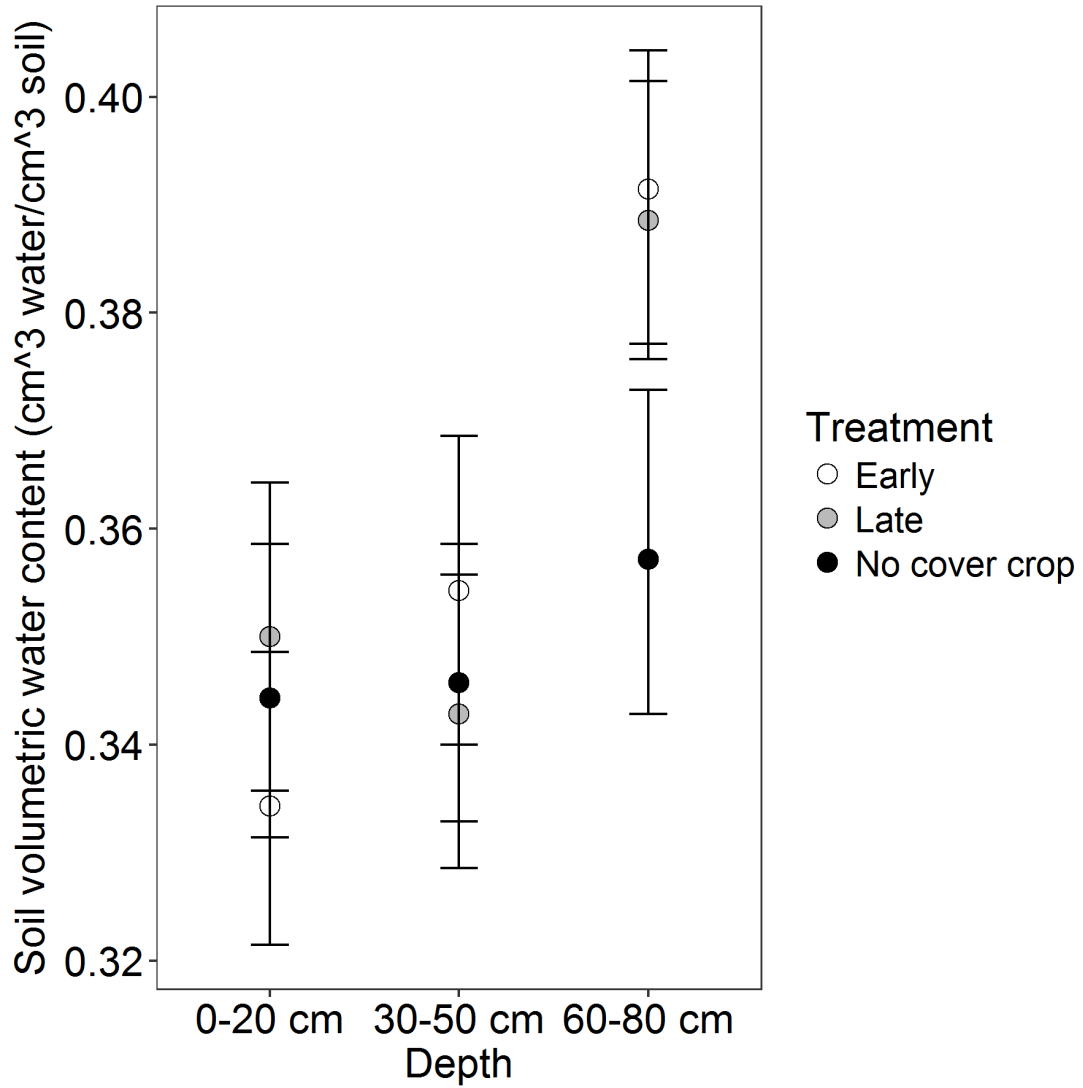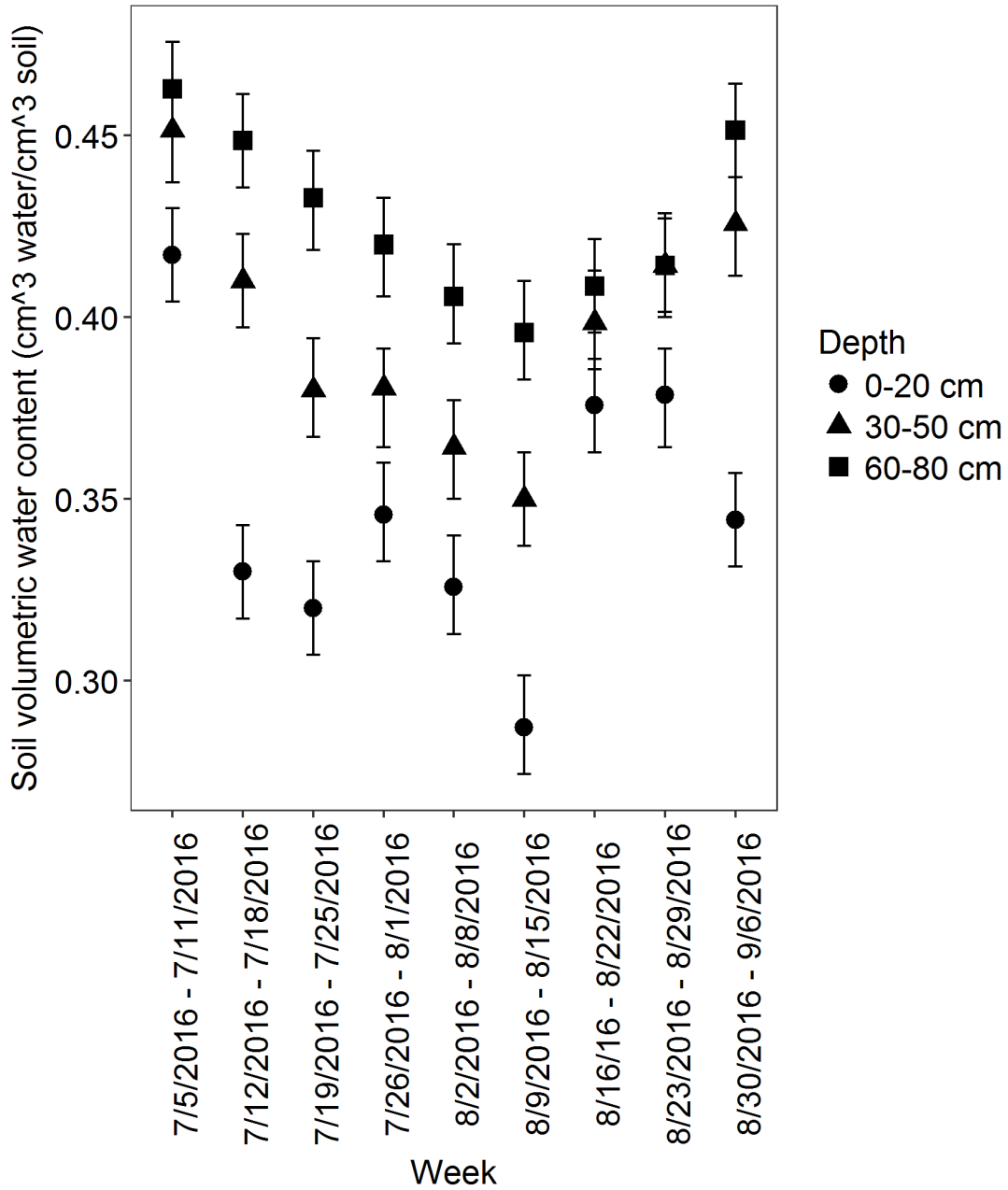
Figure 2.7. 2016 weekly average soil volumetric water content by depth over weeks.

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