ABSTRACT

Title of Thesis:
MANAGING COVER CROPS FOR BETTER
N EFFICIENCY AND SOIL HEALTH

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Winter cover cropping is a major tool that agriculture can use to protect soil and water quality and mitigate climate change. Unlike farmland in the world at large, most Maryland cropland has seen little tillage disturbance and some level of cover cropping for decades. With that background, field experiments on two soils with contrasting textures at the Beltsville Facility of Central Maryland Research and Education Center tested the effects of cover crop management enhancements on nitrogen (N) leaching, soil health indicators, and cover crop N uptake over three years. Two cover crops (sole rye and a mixture of forage radish, crimson clover, and rye) were compared to a control where cover cropping was ceased. The cash crops were corn and soybean grown in rotation. With best nutrient management practices applied, suction lysimeter sampling at 90 cm depth from October through April showed low levels of N leaching in general, but NO_3-N concentrations were significantly lower under cover crops. Overall mean concentrations of NO_3-N were 2.20 mg N/L in the control but 0.43 mg N/L under cover crops. Additionally, soil water samples were digested to determine dissolved organic N (DON) which was found to make up between 44-60% of the total dissolved N in the leaching water. In additional experiments, a small fertilizer N application was made to cover crops to stimulate rapid deep rooting with the goal of accessing soluble N deep in

the profile to increase N capture by more than the amount of N applied. The response to fall N fertilization failed to accomplish this goal and was not related to the surface soil NO_3-N concentration as expected. In spring, cover crops were terminated on three dates from mid-April to mid-May and rye biomass doubled with each extra two weeks it was allowed to grow whether it was in the mix or alone. The effect of cover crops on soil health indicators was evident with increased soil permanganate oxidizable carbon, total soil carbon, lower bulk density, and greater aggregation. These experiments demonstrated that cover crops with enhanced management can have marked effects on an agricultural system already using sustainable practices.

MANAGING COVER CROPS FOR BETTER N EFFICIENCY AND SOIL HEALTH

by

Melissa R. Stefun

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Managing Cover Crops for Better N Efficiency and Soil Health

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Winter cover cropping is a major tool that agriculture can use to protect soil and water quality and mitigate climate change. Unlike farmland in the world at large, most Maryland cropland has seen little tillage disturbance and some level of cover cropping for decades. With that background, field experiments on two soils with contrasting textures at the Beltsville Facility of Central Maryland Research and Education Center tested the effects of cover crop management enhancements on nitrogen (N) leaching, soil health indicators, and cover crop N uptake over three years. Two cover crops (sole rye and a mixture of forage radish, crimson clover, and rye) were compared to a control where cover cropping was ceased. The cash crops were corn and soybean grown in rotation. With best nutrient management practices applied, suction lysimeter sampling at 90 cm depth from October through April showed low levels of N leaching in general, but NO_3-N concentrations were significantly lower under cover crops. Overall mean concentrations of NO_3-N were 2.20 mg N/L in the control but 0.43 mg N/L under cover crops. Additionally, soil water samples were digested to determine dissolved organic N (DON) which was found to make up between 44-60% of the total dissolved N in the leaching water. In additional experiments, a small fertilizer N application was made to cover crops to stimulate rapid deep rooting with the hope that access to soluble N deep in the profile would increase N capture by more than the amount of N applied. The response to fall N fertilization failed to accomplish this and was not related to the surface soil nitrate concentration as expected. In spring, cover crops were terminated on three dates from mid-April to mid-May and rye biomass doubled with each extra two weeks it was allowed to grow whether it was in the mix or alone. The effect of cover crops on soil health indicators was evident with increased soil permanganate oxidizable carbon, total soil carbon, lower bulk density, and greater aggregation. These experiments demonstrated that cover crops with enhanced management can have marked effects on an agricultural system already using sustainable practices.

Introduction

Wayward nitrogen (N) is a major cause of eutrophication in estuaries like the Chesapeake Bay (Pionke et al., 2000); (Testa & Kemp, 2014). In the Chesapeake Bay estuary, land in agricultural production contributes approximately 46% of the N load (Chesapeake Bay Program, 2020). The principal mechanism for N inputs from cropland to the Chesapeake Bay is the leaching of N as nitrate (NO_3-N) and dissolved organic N (DON) to groundwater, and the subsequent flow of groundwater to streams, rivers, and finally the estuary (Phillips & Lindsey, 2003). The dissolved N may originate from unused fertilizer, soil organic matter, organic amendments, leguminous crops, and animal manures. Nitrogen is an expensive essential nutrient for crops, so its leaching is costly to the farmer. Most leaching occurs during the fall and winter when evapotranspiration is lower than precipitation, resulting in the net drainage of water (Thorup-Kristensen et al., 2003); (Meisinger & Delgado, 2002). Most studies on N leaching measure only the nitrate form of dissolved N, but the few studies that also measured the total dissolved N suggest that nitrate comprises only part of the N loss (Jones & Willett, 2006); (van Kessel et al., 2009); (Macdonald et al., 2016); (Salazar et al., 2019); (Liang et al., 2021).

Non-legume cover crops have been shown to remove NO_3-N from the soil profile before the leaching season begins and increase transpiration during the leaching season, thus reducing the volume of water available to carry NO_3-N (Daryanto et al., 2018); (Meisinger & Ricigliano, 2017); (Meisinger et al., 1991). Planting cover crop to remove residual soluble N from the soil profile as soon as possible, even before cash crop harvest, can reduce N leaching (Hirsh & Weil, 2019);(Sedghi & Weil, 2022a). Corn (*Zea mays*) ceases N uptake when it begins to approach maturity which is a month or more before it is ready for harvest (Ciampitti et al., 2013). Even when cover crops are planted as soon after crop harvest as possible, they are unlikely to achieve enough root and shoot growth to remove most of the soluble N from the profile before they enter winter dormancy. Interseeding cover crops into standing cash crops before harvest can reduce N leaching without posing competitive risks for the cash crop. There is evidence that a cover crop that includes several functionally different species provides more benefits to a cropping system over a longer period of time than a single species cover crop (Finney & Kaye, 2017).

The use of cover crops that are capable of vigorous growth in the fall can lead to enhanced N capture (Aronsson et al., 2016). Compared to a pure cereal rye *(Secale cereale L.)* cover crop, brassica species such as forage radish *(Raphanus sativus L.)* planted alone or in a mixture with cereal rye, produced more biomass and took up more N from deep in the soil profile (Dean & Weil, 2009). This combination of deep rooting depth and rapid growth makes radish an optimal N scavenger in fall. However, when the radish is winter-killed it will release soluble N. This N from the decomposing radish could be lost early in spring in sandy soils (Dean & Weil, 2009); (Gieske et al., 2016); (Sedghi & Weil, 2022a), making it beneficial to include winter hardy, N scavenging grass species in a cover crop mix. Brassica and grass species in a mix maintain N in biomass in fall and spring. The timely release of sequestered N for the following crop is another critical function of cover crops. Legume species, such as crimson clover (*Trifolium incarnatum L.*), are the most consistent at providing N to the following crop (Aronsson et al., 2016); (Vyn et al., 1999). Legume species can add additional benefits to a grass-brassica cover crop mix by N fixing in spring and leaving residues with a lower C:N than rye that allows for more rapid N mineralization (Ranells & Wagger, 1997).

Work by Hirsh et al. (2021) examining the synergy of these three species types (brassica, grass, legume) reported that the cover crop mixes influence the spatiotemporal dynamics of soil N in a corn- soy rotation. When comparing a radish monoculture, a rye monoculture and a mix containing radish, rye, and crimson clover, it was the mix that was most effective at balancing capturing N in the fall and releasing N in spring for the following corn crop resulting in a corn yield boost.

More research is needed on the impacts of growing cover crop mixes for an extended duration on N dynamics and soil health in the corn-soybean cropping system that covers much of the cropland in Maryland. Work on the effects of increasing cover crop duration in the field by studying earlier planting dates (Sedghi & Weil, 2022); (Kuo & Jellum, 2002) has shown that N capture in the fall and total amounts of N present in cover crop tissue in the spring increases dramatically with earlier cover crop planting dates. Allowing cover crops to grow longer in the spring (delayed termination) can double or triple the total biomass produced and the atmospheric carbon fixed and added to the soil (Sedghi et al., 2023). However, in the case of winter cereal cover crops delayed termination may result in higher C/N ratio tissue that stimulates immobilization instead of mineralization of N. Work on effects of delayed termination of a cereal rye monoculture cover crop (Schramski et al., 2021); (Krueger et al., 2011) found that rye cover crop terminated within a few days of crop planting usually results in improved N capture but lower N available to the following cash crop due to slow mineralization. Including a brassica and/or a legume species along with a winter cereal may provide the added biomass, carbon, and soil health benefits without the yield-lowering N immobilization (Hirsh et al, 2021). Studies that have compared delayed termination of cover crop mixes to monocultures found that mixes including legumes have similar N capture potential to stands of grass monocultures but also have a lower overall C:N (Lawson et al., 2015); (Odhiambo & Bomke, 2001); (Koehler-Cole et al., 2020). Legumes prevent the C: N in residues from climbing too high for tissue N to be mineralized and made available to the following crop, even when the cover crop is terminated late.

Information on the effects of three individual management practices (early planting, delayed termination, or cover crop mixes) highlights the potential of each management practice to influence N dynamics. this project studied the combination of these three management practices, which is hereafter referred to as "enhanced management." A multi-species cover crop, and a single species cover crop were compared to a no-cover control. Cover crops were planted early and terminated at three dates in the spring. This research measured the effects of these practices on N dynamics, soil properties, and cash crop outcomes.

Project Description

This research studied the impact of enhanced management techniques on the leaching loss of N and the N uptake and yield of subsequent cash crops. Enhanced cover cropping practices included early planting by inter-seeding into standing crops (corn and soybean) before they were harvested, late termination by planting cash crops into living cover crops before termination, and the use of a multispecies mixture (brassica-grass-legume) compared to a single species grass. All variables were studied at two sites with contrasting soils.

We hypothesized that, of the different treatments studied, inter-seeded, late terminated, three-species cover crop would minimize NO_3-N leaching and maximize the availability of N and water to the subsequent crop leading to more efficient N use and higher yields. Additionally, we hypothesized that after three years under enhanced management, soil health indicators would be improved compared to standard or no-cover crops.

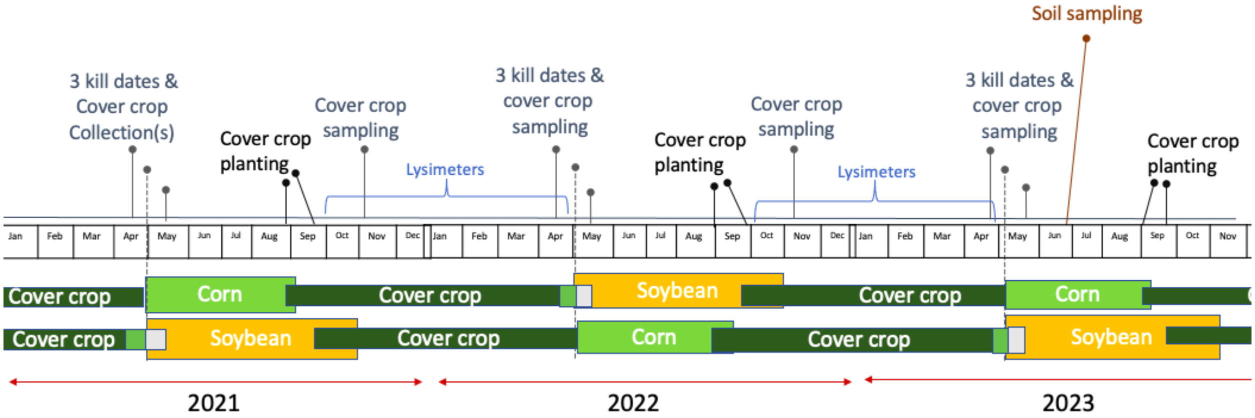


Figure 1. A field timeline showing the duration of cover crops and main crops in the two fields studied. The three cover crop termination dates, cover crop planting dates, sampling dates and lysimeter installation-removal periods are shown and recur annually. The date for soil sampling is shown in 2023 and occurred once.

Chapters

1: Cover Crops Effect on Mineral and Organic Forms of Nitrogen in a Corn-Soy Rotation

2: Cover Crop Management and Adaptive Fertilization for Corn-Soybean Rotation

3. Multi-Species Cover Crop Outperformed Single Species in Biomass and Soil Health in the Short-Term

4: Cover Crop Nitrogen Uptake is Unaffected by Fertilization Even in Depleted Soils

Chapter 1 Cover Crops Effects on Nitrogen Leaching in a Corn-Soybean Rotation

Abstract

Winter cover cropping is a major agricultural tool for protecting soil and water quality and mitigating climate change. Unlike farmland in the world at large, most Maryland cropland has seen little tillage disturbance and had some level of cover cropping for decades. Against that background, field experiments on two contrasting soils near Beltsville, Maryland investigated how cover crop management enhancements affect nitrogen (N) leaching and cover crop N uptake over three years of a corn and soybean rotation. Two cover crops (sole rye and a mixture of forage radish, crimson clover, and rye) were compared to a control where cover cropping was ceased. With best nutrient management practices applied, suction lysimeter macropore water samples collected at 90 cm depth from October through April showed low levels of N leaching in general, but NO_3-N concentrations were significantly lower under cover crops. Additionally, lysimeter water samples were digested to determine total and dissolved organic N (TDN and DON). Overall mean concentrations of NO_3-N were 2.20 mg N/L in the control but 0.43 mg N/L under cover crops regardless of species. Cover crops decreased the estimated mass of leached of NO_3-N with average cumulative leaching season losses being 2.24 kg/ha with cover crops and 9.04 kg/ha without. Cover crops rarely influenced the concentration of TDN or DON, but often changed the proportions among N forms. Under cover crops or no-cover, DON accounted for 60-44% of the TDN in the leaching water with rye>3way> no-cover in the proportion of DON. The mass of TDN lost during a leaching season averaged 18.42 kg/ha with cover crops and 25.98 kg/ha without, but TDN values were different at only $p<0.17$. These results demonstrated that in agricultural systems

already using sustainable practices, cover crops with enhanced management can have marked effects on NO_3-N loss but their ability to reduce TDN loss is less clear.

Introduction

Under suitable growing conditions cover crops are efficient tools to scavenge soil nitrogen (N) not used by the main crop and reduce overall nitrate (NO_3-N) leaching. Without cover crops, a corn (*Zea mays* L.) - soybean (*Glycine max* (L.) Merr.) rotation has no meaningful N uptake for eight to nine months of each year. Soybeans generally receive 50−60% of their N through fixation, and do not remove N from the soil profile in the same amounts as non-legume crops thus, less N is taken up by the cash crop when soybean is grown (Salvagiotti et al., 2008). However, a cover crop's ability to reduce nitrate leaching is influenced by environmental conditions after sowing and the cover crop species' ability to accumulate biomass and take up N. Early cover crop sowing, even preceding cash crop harvest, is essential to maximize growth and support efficient N capture before winter dormancy sets in (Kumar et al., 2023; Sedghi & Weil, 2022b). The most common cover crop in the Mid-Atlantic is a grass species monoculture such as winter wheat (*Triticum aestivum* L.), annual ryegrass (*Lolium perenne* L.) or cereal rye (Secale *cereal* L.) because these are winter hardy and grow well in a variety of conditions (Caswell et al., 2019). However, different species of cover crops may have different growth and N uptake characteristics and may respond differently to growing conditions such as temperature, water supply, and nutrient supply that vary from year to year (Kaye et al., 2019). A diverse mixed species cover crop will increase the chance that, whatever the available resources, sufficient biomass will be produced to provide N scavenging and various ecosystem services, preferably without limiting N availability to the next crop (Chu et al., 2017; Hirsh et al., 2021; Poffenbarger et al., 2015). Giving cover crops longer periods of good growing weather by planting earlier and terminating later, as well as sowing multiple complementary species, can produce a more resilient and productive cover crop (Finney & Kaye, 2017; Hirsh et al., 2021).

The use of cover crop species capable of vigorous growth in the fall can lead to enhanced N capture (Aronsson et al., 2016). Compared to a pure cereal rye *(Secale cereale L.)* cover crop, brassica species such as forage radish *(Raphanus sativus L.)* planted alone or in a mixture with cereal rye, produced more biomass and took up more N from deep in the soil profile (Dean & Weil, 2009). If planted early, this combination of deep rooting depth and rapid growth makes radish an optimal fall N scavenger. However, when the radish is winter-killed it releases soluble N which could be lost by leaching in early spring on sandy soils (Dean & Weil, 2009; Gieske et al., 2016; Sedghi & Weil, 2022a), making it beneficial to include winter hardy, N scavenging grass species in a cover crop mix. Brassica and grass species in a mix maintain N sequestration in fall and spring. The timely release of sequestered N for the following crop is another critical function of cover crops. Legume species, such as crimson clover (*Trifolium incarnatum* L.), are the most consistent at providing N to the following crop (Aronsson et al., 2016; Vyn et al., 1999). Legume species can add additional benefits to a grass-brassica cover crop mix by N fixing in spring and leaving residues with a lower C:N than rye that allows for N mineralization (Ranells & Wagger, 1997).

Work examining the combination of these three cover crop families (brassica, grass, and legume) by Hirsh et al. (2021) examined the cover crop mixes influence on the spatiotemporal dynamics of soil N in a corn-soy rotation. When comparing cover crop treatments of a radish monoculture, a rye monoculture and a mix containing radish, rye, and crimson clover, it was the mix that was most effective at balancing capturing N in the fall and releasing N in spring for the following corn crop resulting in a yield boost some cases.

Even with efficient fertilizer use, it can be difficult to limit N losses in a corn/soybean rotation. The synchronization of cash crop N uptake with inorganic N availability is challenging because the formation of soluble forms of N depends on highly variable factors such as microbial activity, quality of organic matter, temperature, and moisture (Aulakh et al., 1991; Davidson et al., 1991). Ammonification is the conversion of organic N to ammonium-N (NH_4-N), a form that is plant-available and relatively immobile in soils. Another transformation, nitrification, is the conversion of NH_4-N to nitrate-N (NO_3-N), which is plant-available and highly mobile in soils. Together, these two processes are referred to as "mineralization".

Cover crops can improve water quality by sequestering mineral N (NH_4-N, NO_3-N) during periods of particular vulnerability to leaching when cash crops are not taking it up and evapotranspiration is lower than precipitation (Blanco-Canqui, 2018). Nitrate leaching is highly responsive to precipitation because of NO_3-N mobility with drainage water (Meisinger & Ricigliano, 2017; Fraser et al., 2013). Soil texture, weather, and cover crop presence all influence soil moisture which, in turn, impacts N forms and movement (Zhu et al., 2018; Basche & DeLonge, 2019). In this study, three cover crop treatments are compared in two fields with contrasting soil textures, during two years with contrasting weather conditions.

The three cover crop treatments were: 1) no-cover crop winter fallow without weed control (NC), a mixture of forage radish (*Raphanus sativus* L.), crimson clover (*Trifolium incarnatum* L.), and cereal rye (*Secale cereale* L.) (3WAY), and a monoculture cover crop of cereal rye (RYE). These were integrated into a corn/soy rotation in two fields with contrasting soil textures, one very sandy, and the other dominated by silt and clay.

Over two years from 2021-2023, we investigated the effects of the three cover crop treatments on,

- Concentrations of NO_3-N, NH_4-N, and dissolved organic-N (DON) in 90 cm deep soil macropore water during the 7-month leaching season between the harvest of one cash crop and the planting of the next.
- Mass of NO_3-N and total N leached during the 7-month leaching season
- The relative contribution of NO_3-N, NH_4-N, and DON to the concentration of total dissolved N (TDN) in leaching water

Materials and Methods

Sites and Management

We conducted field experiments at two sites with contrasting soils at the Beltsville Facility of the University of Maryland Central Maryland Research and Education Center (CMREC) (39°00'46.0"N 76°49'35.7"W). One site (called Sandy) was dominantly Downer and Hammonton soils with loamy sand to sandy loam textures throughout the profile, while the other site (called Silty) was dominated by Christiana and Russet soils with silt loam A horizons over silty clay loam subsoils. Both sites had a history of 35 years of management with no-till farming of corn, soybean, and wheat, with cover crops included whenever possible during the last 20 years of that period.

Plots were arranged in a randomized complete block (RCB) design with split-split plots and four replicates at each of the two sites. The main plots (55m by 27m) comprised of two cash crops (corn or soybean in rotation), planted in 72 cm wide rows. The subplots (55m by 9m) comprised three cover crop treatments, NC, RYE, and 3WAY, and the sub-subplots (18m by 9m) were assigned cover crop termination times EARLY, MID, and LATE, ahead of soybean and N fertilization rates during corn (Table 1-1). Corn was fertilized with three rates, ZERO, MED, and

HIGH. The HIGH rate of N was applied according to MDA nutrient management guidelines at of 1 kg N /56 kg of anticipated yield (Maryland Nutrient Management Manual I-B1 P1-15, Annapolis, MD). The MED N rate (medium) was a more conservative application of about 0.73 kg N/56 kg grain. Corn yields at each field in 2021 were lower with MED than with HIGH so both rates were increased in 2022 with a greater increase at the silty field (Table 1-2).

Cover crops were overseeded into standing corn or soybean crops with a Spra Coupe Sprayer (4640, Duluth GA) fitted with an Orbit Air Seeder (62 series 62DS12F, Owatonna, MN) and drop tubes. Seeding rates were 112 kg/ha cereal rye in RYE and 4.5, 17, 84 kg/ha for the radish, crimson clover, and rye, respectively, in 3WAY. Corn and soybean were no-till planted with John Deere MaxEmerge no-till planter (Moline, IL), in 72 cm wide rows with a target of 74,132 and 395,368 viable seeds per hectare, respectively. All plots were managed with no-till techniques, so tillage was not performed during the study. Cover crops were terminated on the dates shown in Table 1-1 by spraying herbicides. Where corn was planted cover crops were also roller crimped.

Table 1-1 Cover crop management at two sites from 2020-2023.

Site	Cover crop planting date into corn	Cover crop planting date into soybean	Cover crops fall sampling date	Cover termination date*		
				EARLY	MID	LATE
Sandy	6/26/20	9/7/20	12/10/20	4/14/21	5/13/21	5/21/21
Silty	6/27/20	9/7/20	12/13/20	4/14/21	5/13/21	5/21/21
Sandy	8/30/21	9/16/21	11/13/21	4/10/22	5/10/22	5/21/22
Silty	9/1/21	9/16/21	11/14/21	4/10/22	5/10/22	5/21/22

Sandy	9/1/22	9/14/22	12/10/22	4/12/23	5/10/23	5/25/23
Silty	9/12/22	9/14/22	12/12/22	4/12/23	5/10/23	5/25/23

** Cover crop termination was by herbicide (In Corn: Glyphosate, 2.34 L/ha, ai 0.96 L/ha, saflufenacil 0.15 L/ha, ai 0.017 L/ha, methylated seed oils 0.710 L/ha. In soybean: Glyphosate, 2.34 L/ha, ai 0.96 L/ha, 2,4-D 1.75 L/ha, ai 0.98 L/ha). Spring cover crop biomass sampling date was 1-2 days before termination.*

Table 1-2 Management of corn and soybean from 2021-2023 at two sites.

Site	Year	Corn Planting	Corn Harvest	Corn side-dress N rates, kg/ha			Soybean Planting	Soybean Harvest
				ZERO	MED	HIGH		
Sandy	2023	May 10th	Sept 26th	0	112	179	May 10th	Oct 10th
Silty	2023	May 9th	Sept 9th	0	123	202	May 9th	Oct 13th
Sandy	2022	May 10th	Sept 19th	0	112	179	May 10th	Oct 25th
Silty	2022	May 12th	Sept 22nd	0	123	202	May 12th	Oct 28th
Sandy	2021	May 4th	Sept 22nd	0	84	168	May 6th	Oct 20th
Silty	2021	May 4th	Sept 20th	0	84	168	May 6th	Oct 19th

Weather

Daily temperature, precipitation, and 30-year averages were determined using Oregon State University software (*PRISM*, 2004) (**Error! Reference source not found.**).

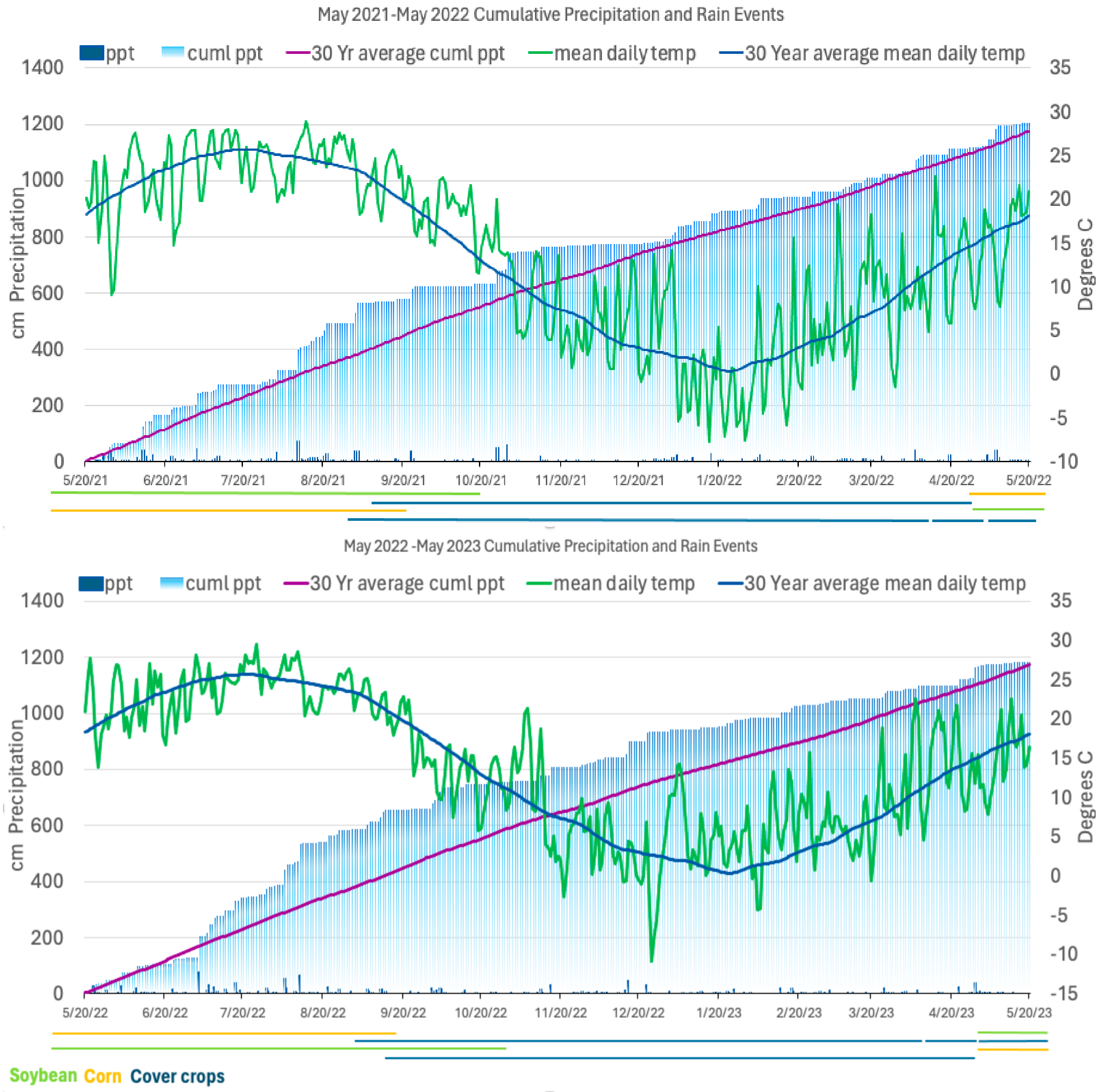


Figure 1-1 Site daily precipitation, cumulative precipitation, mean daily temperature compared to the 30-year average for cumulative precipitation and mean daily temperature and growing season periods for crops and cover crops. Upper panel is for 2021-2022 and lower panel is for 2022-2023. Weather data sourced from PRISM, Oregon State University. Ppt = ppt = precipitation; cuml ppt = cumulative precipitation.

The study took place during two contrasting leaching seasons. The fall of 2021 was far drier than normal and the precipitation that came was uneven with weeks or months without meaningful rain. Cumulative rainfall caught up to the 30-year average for three weeks in the fall but was about 10 cm below the average for the remainder of the sampling period. The mean daily temperatures did not deviate much from the 30-year average, but the fall and first quarter of winter were warmer than normal. The remaining winter temperatures were colder than normal, while the spring was similar to the average. Winter temperatures were cold enough for the radish in the 3WAY to winter-kill. The fall of 2022 displayed different conditions to the previous year. Rain was plentiful and frequent, largely staying in step with the average into the winter. The spring was drier than normal with the cumulative precipitation staying about 5 cm below the average. Mean daily temperatures were well below the average in the early fall. However, temperatures were above average for most of the winter and spring. Winter temperatures were still cold enough for the radish to winter-kill.

Porewater N Concentrations

During the leaching season from October to May, porewater leachate samples were collected biweekly under 80 kPa tension from ceramic-tipped suction lysimeters and analyzed for nitrate, ammonium and total dissolved N.

The suction lysimeters were made of butyrate tubes (22-mm outer diameter) with 100-kPa air entry ceramic tips (Irrometer, Inc. Riverside, CA) and were installed to 90 cm deep at two representative locations in each cover crop x main crop sub-subplot sampled. Lysimeters were installed in the sub-subplot where the previous corn crop received the HIGH N rate or where the cover crop preceding the previous soybean crop was terminated late. They were installed as soon as possible after cash crop harvest to obtain soil macro-porewater samples from the bottom of the crop root zone beginning in Fall. Before installing each lysimeter, a pilot hole of the same diameter was made using a drop hammer device, and 200 ml of water–subsoil slurry (using soil material from 70-90 cm in the same field) was poured down the hole to ensure good contact between the ceramic tip and the soil. The upper 5–10 cm of soil around each lysimeter was sealed with bentonite to prevent preferential flow. A 75-to-85-kPa vacuum was applied for about 24 hours to collect porewater samples every 2 weeks throughout the cover crop growing season. Porewater samples were pumped into 50 mL centrifuge tubes, kept cold during transport and frozen until analyzed.

After thawing, porewater samples were analyzed for pH and electrical conductivity (EC) using a Horiba LAQUAtwin Model PH-11 Compact Water Quality pH Meter and Horiba LAQUAtwin EC-33 Compact Conductivity Meter, then filtered through a 0.45 μm acetate filter membrane, and frozen again until they could be analyzed colorimetrically for nitrate, ammonia and TDN. The concentration of NO_3-N was measured by the salicylic acid colorimetric method (Cataldo et al., 1975), the concentration of NH_4-N was measured by the salicylate colorimetric method (Forester, 1995), and the total dissolved N (TDN) concentration was measured as for nitrate-N after persulfate digestion (Hosomi & Sudo, 1986). The dissolved organic nitrogen was calculated by subtracting the mineral N fractions from the total N:

$$DON = TDN - (NO_3\text{-}N + NH_4\text{-}N) \qquad \text{(eq. 3)}$$

Mass Balance Estimate

The water drainage volume (D, L/m2/day) was estimated from precipitation (P, mm/day), evapotranspiration (ET, mm/day) and runoff (R, mm/day) as:

$$D = P - ET - R \qquad \text{(eq. 4)}$$

Data for precipitation, mean daily temperature, and cloud transmittance for the site locations was sourced from (PRISM 2004). Runoff volume was measured by erosion weirs collecting from 0.31 m^2 during the study periods at the silty site. No rainstorms that occurred during the study period were intense enough to cause runoff at the sandy site and runoff was considered zero when calculating drainage volume for the sandy site. Evapotranspiration was accessed through DTN (Bloomington, MN) which is estimated using the Hargreaves equation (Hargreaves & Samani, 1985). For each sampling date the average measured concentration of NO_3-N was multiplied by the water drainage volume for 1 m^2 and converted to kg/ha of NO_3-N mg N/L x L/m^2 x m^2/ha x kg/mg = kg N/ha lost per day. For each sampling date the average NO_3-N concentration measured was assumed for the drainage volume calculated between the day midway between the current and previous sampling date and the day midway between the current and subsequent sampling date.

Statistical Analysis

Data were analyzed using R Studio version 1.2.5042, using R packages pacman, lme4, and emmeans. The Shapiro-Wilk test was used to assess normality, and power transformations were applied to satisfy assumptions of normality and homogeneity of variances. If data could not be transformed to a normal distribution, non-parametric tests Kruskal-Wallis rank sum test and pairwise comparisons using Wilcoxon rank sum test with continuity correction with Benjamini-Hochberg p value adjustment method. Soil porewater data was never able to be transformed to a normal distribution. The data was unbalanced because not every lysimeter produced a sample on every sample date. Therefore, only non-parametric tests were used for N concentration and mass amounts of N in drainage. Crop and cover crop biomass data were analyzed with analysis of variance (ANOVA) and post-hoc analysis with Tukey Honestly Significant Difference (HSD). The effect of Year and Block nested within Field were considered as random effects and fall cash crop and cover crop treatment were considered as fixed effects.

Results and Discussion

N Porewater Concentrations

Concentrations of NO_3-N observed during the two leaching seasons were extremely low and often near the lower limit of detection (Figure 1-2). In fall 2021 concentrations of NO_3-N in leachate across treatments and soils were extremely low with no significant differences between treatments. The very low levels of nitrate-N in soil water were likely due to the low soil moisture and precipitation during fall 2021. As rainfall and soil moisture increased during the winter and spring, NO_3-N concentrations increased substantially for the NC treatment but remained significantly lower under the two cover crop treatments.

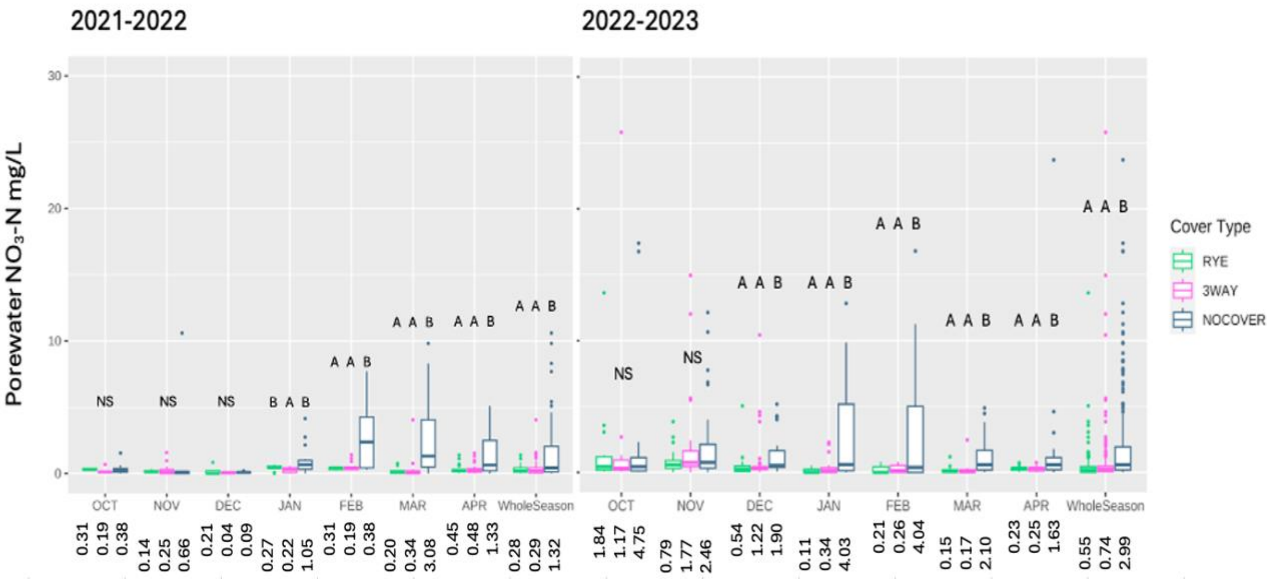


Figure 1-2 Concentration of NO_3-N in soil water measured at 90 cm from October-April in 2021-2022 on the left and 2022-2023 on the right. The center bar in the box represents the median and the mean concentrations are displayed under each box. Within each month, cover crop types with different letters are significantly different. Krusker-Wallis and Mann-Whitney significance of $p< 0.05$. N=968

During fall 2022 concentrations of NO_3-N were higher than the previous year, but still low compared to most mid-Atlantic cropland sites reported in the literature (Staver & Brinsfield, 1998; Ritter et al., 1998; Meisinger & Ricigliano, 2017; Sedghi et al., 2023). The fall of 2022 saw ample precipitation which likely drove more NO_3-N loss relative to the previous year. The cooler temperatures in the early fall of that year led to very poor radish establishment and growth with average early December above and below-ground biomass 65.6 and 65.0 kg/ha at the silty and sandy sites, respectively. From December 2022 through April 2023 NO_3-N concentrations remained very low under RYE and 3WAY at both sites while significantly higher concentrations were measured under NC at both sites. Lysimeters were placed in the HIGH N corn plots and in soybean plots in the late termination treatment, Table 1-2. The HIGH N rates were 168 kg/ha at both the silty and sandy sites in 2021, and were increased to 179 kg/ha at the sandy site and 202 kg/ha at the silty site in 2022 as the corn yields growth curve in 2021 did not plateau between the MED and HIGH rates suggesting that there could be higher yields with more N. The yields were higher in 2022 vs 2021 at the sandy site but lower in 2022 compared to 2021 at the silty site. This was likely because July of 2022 was very wet, and the silty clay loam soil of the silty field does not drain excess moisture as readily as the sandy loam soil at the sandy site. The excess rain did not benefit the corn and fostered higher weed growth at the silty site, but not at the sandy site. Though the N application rate was increased from 2021-2022 it is unlikely that it was the reason for higher NO_3-N concentrations in leachate in 2022 since the rate was not greatly increased, +11 kg N/ha at the sandy site and +34 kg N/ha at the silty site. The sandy site, where the NO_3-N losses were on average higher in the fall of 2022 (Table 1-3), saw the higher corn yields (Table 2-5), when compared to the silty site that had lower NO_3-N concentrations compared to the sandy site, suggesting that additional side-dress N was used by the crop rather than leached. The soils at the sites are low OM soils with the sandy field average of 1.42 g/kg and the silty 2.37 g/kg from 0-15 cm depth (Table 3-1). Not much N is released by decomposition with such low OM levels and may be a part of why NO_3-N concentrations porewater are so low in this study.

Overall, NO_3-N loss during the leaching season was very low, particularly in 2021-2022. Average season or annual NO_3-N concentrations never exceeded drinking water standards, with or without cover crops (WHO 11.3 NO_3-N mg L^{-1} and EPA 10 mg/L of NO_3-N). Cover crops lowered concentrations of NO_3-N to nearly undetectable levels even in an already low N system. Average NO_3-N concentrations for each of the two leaching periods studied were 0.29 and 0.45 mg/L for RYE, 0.33 and 1.30 mg/L for 3WAY, and 1.44 and 2.91 mg/L for NC. These concentrations are similar to what other researchers have measured for early planted cover crops established before cash crop harvest with conservative N rates used on corn. Sedghi et al. (2023) found concentrations averaged over 18 sites years to be 11.8 NO_3-N mg/L with no-cover crop and 1.95 NO_3-N mg/L with a brassica-legume-cereal mixture cover crop planted before cash crop harvest. Kaspar et al. (2012) found an average of 8.9 NO_3-N mg /L with an oat cover crop planted before corn and soybean harvest compared to the fallow control which had an average concentration of 12 NO_3-N mg/L. Compared to NO_3-N concentrations in later planted cover crops, these levels are quite low. When cover crops were planted after crop harvest Sedghi et al. (2023) measured an average of 2.68 NO_3-N mg/L over three years while Kaspar et al. (2012) reported 6.2 NO_3-N mg/L over 5 years, and Meisinger & Ricigliano (2017) found 7.45 NO_3-N mg/L over 3 years.

Total Dissolved N Concentrations in Porewater

The effect of cover crops on leaching water N concentrations was less pronounced for TDN than for NO_3-N. The overall mean concentrations of TDN in soil pore water across all years and sites were 5.34 (a), 5.61 (ab), and 7.87 (b) mg N/L for 3WAY, RYE, and NC, respectively with the letters in parentheses indicating significant differences at a Wilcox p value <0.02. Total Dissolved

N concentration did not differ significantly by cover crop within any single month in either of the two leaching seasons Figure 1-3. When only data for the 2021-2022 leaching period is considered, there was no effect of cover crop on TDN concentration, but in 2022-2023 TDN concentrations were lower under 3WAY and RYE than under NC. As with the NO_3-N concentrations, TDN was higher in the leaching season of 2022-2023 compared to 2021-2022.

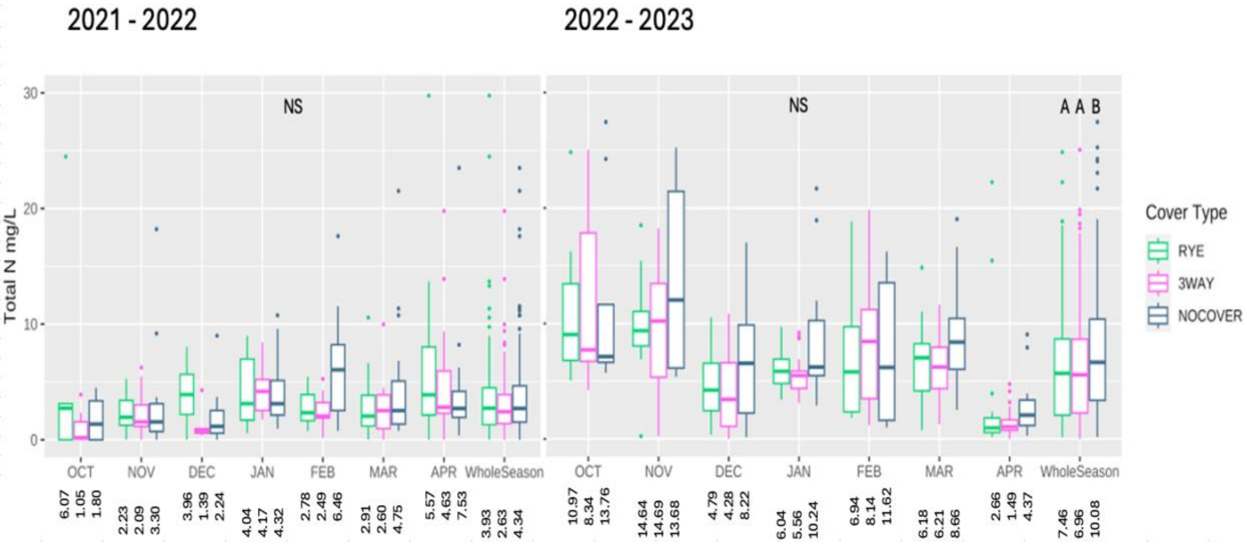


Figure 1-2 Concentration of total dissolved N in soil water measured at 90 cm from October-April in 2021-2022 on the left and 2022-2023 on the right. The center bar in the box represents the median and the mean concentration is displayed beneath each box. Within each month, cover crop types with different letters are significantly different. The only significant difference displayed was for the whole season 2022-2023, otherwise total N concentration did not differ by cover crop. Krusker-Wallis and Mann-Whitney significance of $p< 0.05$. N=681

Estimated N Mass Leached

The mass of NO_3-N and TDN leached was overall higher in the 2022-2023 season compared to the 2021-2022 season. This is likely due to the higher amount of precipitation and therefore higher net drainage volume in the 2022-2023 period. In the same year, the silty site typically had lower NO_3-N losses compared to the sandy site, but the total N loss was not consistently impacted by the soil texture. The NC control plots lost the largest mass of NO_3-N in every year at every site, but NO_3-N loss was essentially the same for 3WAY and RYE for a given site-year. The estimated annual NO_3-N losses from NC averaged across both soils (14.9 kg N/ ha/y in 2021-2022 and 12.7

kg N/ ha/y in 2022-2023) were within the range reported in other studies measuring leaching from non-cover-cropped agricultural systems. For example, Tonitto et al. (2010) measured NO_3-N leached from an organic corn and soybean rotation using large gravimetric lysimeters over 10 years. They reported a mean of 11.5 kg/ha of NO_3-N leached each year but with high year-to-year variability (yearly NO_3-N losses ranged 10-fold from 3.4-35.5 kg/ha).

*Table 1-3 The estimated mass of N and NO_3-N lost to leaching during the two study periods October-April in 2021-2023. The percent reduced is the amount the cover crops prevented from leaching compared to NC. Cover crops mean NO_3-N mass with a letter are significantly different from NC at the same site during the same year with a Krusker- Wallis, Wilcoxon p value **= $p < 0.005$, ***= $p<0.0001$, † = p= 0.070*

Site	**Cover crop**	**2021-2022 leaching season**				**2022-2023 leaching season**			
		NO_3-N, kg/ha	**% reduced**	**Total N, kg/ha**	**% reduced**	**NO_3-N, kg/ha**	**% reduced**	**Total N, kg/ha**	**% reduced**
Sandy	RYE	0.99b***	87%	8.36a	39%	4.72b***	73%	30.88a	23%
	3WAY	0.97b***	87%	8.98a	35%	6.86b***	61%	31.65a	21%
	NC	7.40a	-	13.72a	-	17.54a	-	40.18a	-
Silty	RYE	0.87b**	75%	11.72a	27%	0.99b***	87%	23.37ab	31%
	3WAY	1.07b**	69%	11.20a	30%	1.47b***	81%	21.22b†	37%
	NC	3.47a	-	16.07a	-	7.76a	-	33.93a	-

Averaged across the entire two-year study, we found that the cover crop treatments reduced estimated NO_3-N loss by 78% compared to losses under NC. This magnitude of loss reduction is similar to modeling estimates (85% reduction) (Hively et al., 2020) and field studies (82-84%) (Sedghi et al., 2023) undertaken on early-planted cover crops in Maryland. The smallest reduction (61%) in NO_3-N leaching was observed in 3WAY at the sandy site in 2022-2023, likely due to a combination of cool conditions in fall that resulted in exceptionally poor radish growth, paired with a coarse sandy soil and relatively high precipitation. For TDN, the reduction in leaching loss by cover cropping was much less (21-39%) than the reduction of NO_3-N leaching loss. Though there was a pattern of a TDN loss reduction with cover crops, the only significant difference during a specific site year ($p<0.07$) was 3WAY being lower than NC at the silty site. The difference between the N loss of the cover crop treatments and NC in these estimates are driven by the differences in the measured concentrations in leachate- not by cover crop influence on the field hydrology. Cover crops may change soil hydrology by increasing water infiltration, transpiration during active growth, and mulching soil after termination. Research on the subject supports the theory that cover crops can not only reduce leaching of NO_3-N and other nutrients but also reduce intensive drainage that leads to N loss (Gabriel et al., 2019, 2021; Haruna et al., 2018; Thorup-Kristensen et al., 2003). The cover crop effect on soil drainage during the leaching seasons was not measured in this study so this estimate of N mass lost is conservative. The gap between the N lost under NC compared to the cover crop treatments would likely be wider if the cover crop hydrological effect was considered.

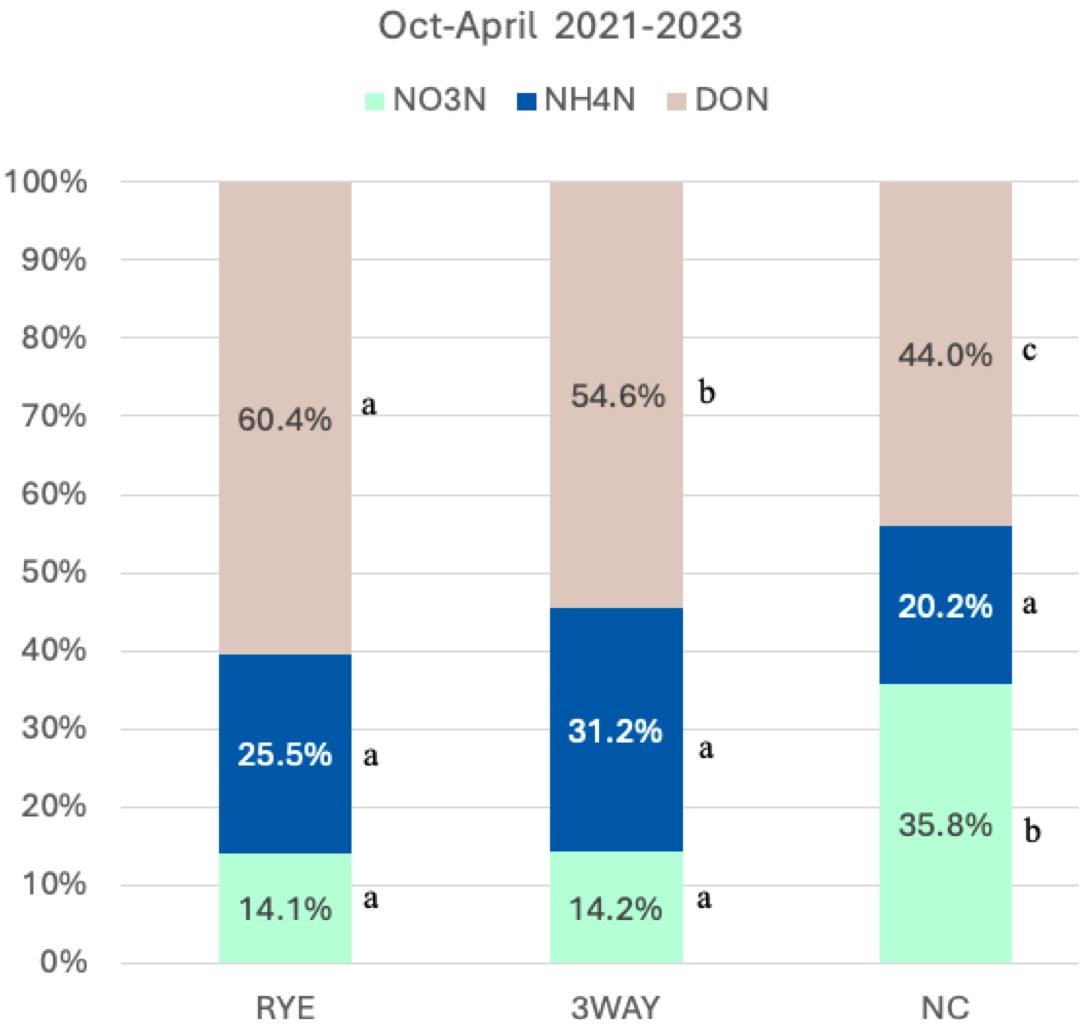


Figure 1-3 The average fraction of each N form relative to the TDN averaged across two seasons and two sites. Within each N form, a mean N form's share of TDN under each cover crop is followed by a different letter if it is significantly different with a Krusker-Wallis, Wilcox $p<0.05$. Differences in the share that NH_4-N made of TDN under the cover crop treatments were non-significant. N=681

The proportions of the dissolved NO_3-N, NH_4-N, and Organic N forms were significantly affected by cover crop treatments, Figure 1-3. Both the NO_3-N concentrations (discussed above) and the proportion of NO_3-N in the TDN were consistently lower under the RYE and 3WAY compared to NC. Conversely, the DON proportion was higher for RYE than for 3WAY, which was higher than NC. The proportion of NH_4-N was similar under all the three cover treatments. Regardless of cover treatment, DON comprised the largest portion of the TDN. This is a departure from observations by others. In a soil column lysimeter study by Salazar (2019), the DON share of TDN was 6.74%

under corn with a grass cover crop and 2.59% with a legume cover crop. The highest observed DON proportion of TDN was 35.47% from an unfertilized continuous grass cover crop and 8.40% from the legume cover crop. In a study on soils with various textures and land management characteristics but no manure application in the UK, Shepherd et al. (2001) reported DON losses in soil drainage made up 8% of total N lost on average. A review of DON loss from agricultural settings by van Kessel et al. (2009) reported that on average DON made up 26% of total N lost by leaching. The higher percentage of DON in this study may partly result from the very low concentrations of mineral N, particularly NO_3-N, rather than an abundance of DON. van Kessel et al. (2009) reported leachate concentrations of DON in the UK ranged between 0.2 - 3.5 mg N/L but the highest concentration in leachate collected from a depth of 90 cm, like in the present study, was 2.6 mg N/L. Salazar (2019) found DON concentrations between 0.78-6.16 mg N/L in leachate from corn and cover crops grown in column lysimeters with the grass cover crop tending to produce the higher DON concentrations compared to a legume cover crop. This is similar to the DON concentrations we observed which averaged 4.49, 3.83 and 4.91mg N /L for RYE, 3WAY, and NC, respectively. For a given site-year, there was no significant effect of cover crop treatment on DON concentrations, Table 1-4.

Table 1-4 Average DON concentrations at two sites and two leach seasons 2021-2022, 2022-2023 under cover crop treatments. Mean concentration within the same year site and site followed by a different letter are significantly different. RYE is compared to NC and 3WAY is compared to NC. Mean DON concentration under the two cover crops is compared to the NC with Krusker- Wallis, Wilcoxon p value given.

		2021-2022		2021-2023	
Site	Cover crop	DON mg/L	*p* value	DON mg/L	*p* value
Sandy	RYE	2.02a	*0.87*	6.08a	*0.84*
Sandy	3WAY	1.31a	*0.83*	5.57a	*0.85*
Sandy	NC	1.42a	-	5.61a	-
Silty	RYE	3.33a	*0.39*	6.06a	*0.78*
Silty	3WAY	2.55a	*0.86*	5.71a	*0.48*

Silty	NC	4.10a	-	7.95a	-

The differences we observed in the composition of the TDN under RYE and 3WAY compared NC makes it more clear why concentrations and cumulative mass of TDN leached was not as sensitive to the cover crops as was NO_3-N. While the concentration of NO_3-N is lower with cover crops, the DON levels are statistically similar, though typically slightly lower under cover crops (Table 1-4). Since DON makes up the largest portion of total N, TDN was generally only slightly lower under cover crop treatments than NC. Cover crops that achieve sufficient biomass are known to increase soil organic matter (SOM) (Blanco-Canqui, 2021;Jian et al., 2020). As SOM includes organic N, the increase of SOM might be expected to also increase leaching of DON. Our study provided no evidence of this effect, however. More research is needed to define the relationship cover crops have with DON leaching and ultimately with all leachable N.

It is known that estuarine bacteria that can cause eutrophication can metabolize DON, though the bioavailability varies by molecular weight (Seitzinger & Sanders, 1997), size (Li et al., 2020), and complexity (Fiedler et al., 2015). Research by O'Neil et al. (2012) showed that an algal bloom on the US East Coast was likely related to increased DON concentrations. While much the role that DON and specifically, DON of agricultural origin, plays in eutrophication is not well-understood, it is clear that DON should be considered when studying N loss from agricultural lands. This experiment demonstrates that DON is a relevant form of N lost from agricultural systems and should be included in nitrogen budgets and land management strategies.

Conclusion

The agricultural system in the current study is low in soil organic matter and soil mineral N. Still, the NO_3-N loss was consistently lower under cover crops whether multi-species or single species.

The use of cover crops in this study did not provide a drag on crop yields, but rather occasionally enhanced crop yields under certain weather conditions. Even where N was applied to corn at traditional rates of 1 kg N per 56 kg expected grain yield, nitrate leaching was almost nonexistent where either cover crop treatment was implemented and significantly higher where no cover crop was planted. Though the statistical confidence level for the cover crops effect on the concentration of TDN was lower than for NO_3-N, the average TDN concentration tended to be lower under cover crops compared to NC with the majority of the TDN being DON. Even in an agricultural system already using sustainable practices like no-till and precise nutrient management, multi-species and single species cover crops still have functions to offer in reducing NO_3-N loss, though their effect on TDN losses is unclear. The question of how cover crops affect the leaching of TDN therefore requires additional research attention.

Chapter 2 Cover Crop Management and Adaptive Fertilization for Corn-Soybean Rotation

Abstract

Winter cover cropping is a major agricultural tool for protecting soil and water quality and mitigating climate change. Unlike farmland in the world at large, most Maryland cropland has seen little tillage disturbance and had some level of cover cropping for decades. Against that background, field experiments on two contrasting soils near Beltsville, Maryland investigated how cover crop management enhancements affect cover crop N uptake and crop yields over three years of a corn and soybean rotation. Two cover crops (sole rye and a mixture of forage radish, crimson clover, and rye) were compared to a control where cover cropping was ceased. Corn was fertilized at three rates of N and soybean had cover crops terminated at three dates in spring. Fall cover crop biomass and N content were always higher for the species mixture than for the rye and the weeds in the no-cover which did not differ significantly. Soil porewater NO_3-N concentrations were low overall and largely unaffected by prior crop type. Cover crops had limited effect on corn and soybean yields but often improved yields when influential. Soybean yields were entirely unaffected by the cover crop termination date and yields ranged from 4,236 -2,287 kg/ha depending on site-year. The first year's corn yield benefitted from the highest N rate, after which the high and intermediate N rates were increased slightly for the remaining two years, and the yield benefit disappeared. Corn yields ranged from 12,972-7,543 kg/ha depending on the site-year. Findings indicate that competitive yields are achievable with advanced cover crop management and conservative fertilization.

Introduction

Under suitable growing conditions cover crops are efficient tools to scavenge soil nitrogen (N) not used by the main crop and reduce overall nitrate (NO_3-N) leaching. Without cover crops, a corn (*Zea mays* L.) - soybean (*Glycine max* (L.) Merr.) rotation has no meaningful N uptake for eight to nine months of each year. Soybeans generally receive 50−60% of their N through fixation, and do not remove N from the soil profile in the same amounts as non-legume crops thus, less N is taken up by the cash crop when soybean is grown (Salvagiotti et al., 2008). However, a cover crop's ability to reduce nitrate leaching is influenced by environmental conditions after sowing and the cover crop species' ability to accumulate biomass and take up N. Early cover crop sowing, even preceding cash crop harvest, is essential to maximize growth and support efficient N capture before winter dormancy sets in (Kumar et al., 2023; Sedghi & Weil, 2022b). The most common cover crop in the Mid-Atlantic is a grass species monoculture such as winter wheat (*Triticum aestivum* L.), annual ryegrass (*Lolium perenne* L.) or cereal rye (Secale *cereal* L.) because these are winter hardy and grow well in a variety of conditions (Caswell et al., 2019). However, different species of cover crops may have different growth and N uptake characteristics and may respond differently to growing conditions such as temperature, water supply, and nutrient supply that vary from year to year (Kaye et al., 2019). A diverse mixed species cover crop will increase the chance that, whatever the available resources, sufficient biomass will be produced to provide N scavenging and various ecosystem services, preferably without limiting N availability to the next crop (Chu et al., 2017; Hirsh et al., 2021; Poffenbarger et al., 2015). Giving cover crops longer periods of good growing weather by planting earlier and terminating later, as well as sowing multiple complementary species, can produce a more resilient and productive cover crop (Finney & Kaye, 2017; Hirsh et al., 2021).

The use of cover crop species capable of vigorous growth in the fall can lead to enhanced N capture (Aronsson et al., 2016). Compared to a pure cereal rye *(Secale cereale L.)* cover crop, brassica species such as forage radish *(Raphanus sativus L.)* planted alone or in a mixture with cereal rye, produced more biomass and took up more N from deep in the soil profile (Dean & Weil, 2009). If planted early, this combination of deep rooting depth and rapid growth makes radish an optimal fall N scavenger. However, when the radish is winter-killed it releases soluble N which could be lost by leaching in early spring on sandy soils (Dean & Weil, 2009; Gieske et al., 2016; Sedghi & Weil, 2022a), making it beneficial to include winter hardy, N scavenging grass species in a cover crop mix. Brassica and grass species in a mix maintain N sequestration in fall and spring. The timely release of sequestered N for the following crop is another critical function of cover crops. Legume species, such as crimson clover (*Trifolium incarnatum* L.), are the most consistent at providing N to the following crop (Aronsson et al., 2016; Vyn et al., 1999). Legume species can add additional benefits to a grass-brassica cover crop mix by N fixing in spring and leaving residues with a lower C:N than rye that allows for N mineralization (Ranells & Wagger, 1997).

Work examining the combination of these three cover crop families (brassica, grass, and legume) by Hirsh et al. (2021) examined the cover crop mixes influence on the spatiotemporal dynamics of soil N in a corn-soy rotation. When comparing cover crop treatments of a radish monoculture, a rye monoculture and a mix containing radish, rye, and crimson clover, it was the mix that was most effective at balancing capturing N in the fall and releasing N in spring for the following corn crop resulting in a yield boost some cases.

The Maryland Nutrient Management Manual recommends applying 1.0 lb N/ bushel of expected corn grain yield up to 250 bu/acre, or 1 kg of N per 56.5 kg expected yield (Maryland Nutrient Management Manual I-B1 P1-15, Annapolis, MD). While helpful as a guideline, an N rate chosen purely based on expected yield is unlikely to be the economic or environmental optimum rate for a particular field. One way to improve nutrient application rates on the field level are adaptive management techniques that use continuous systematic assessment from year to year while comparing yields using two or more different N rates (Morris et al., 2018). If the higher N rate provides no increase in corn grain yield on average, then the lower rate can be used to produce comparable yields but with lower input costs and less impacts to the surrounding environment.

Even with efficient fertilizer use, it can be difficult to limit N losses in a corn/soybean rotation. The synchronization of cash crop N uptake with inorganic N availability is challenging because the formation of soluble forms of N depends on highly variable factors such as microbial activity, quality of organic matter, temperature, and moisture (Aulakh et al., 1991; Davidson et al., 1991). Ammonification is the conversion of organic N to ammonium-N (NH_4-N), a form that is plant-available and relatively immobile in soils. Another transformation, nitrification, is the conversion of NH_4-N to nitrate-N (NO_3-N), which is plant-available and highly mobile in soils. Together, these two processes are referred to as “mineralization”.

Cover crops can improve water quality by sequestering mineral N (NH_4-N, NO_3-N) during periods of particular vulnerability to leaching when cash crops are not taking it up and evapotranspiration is lower than precipitation (Blanco-Canqui, 2018). Nitrate leaching is highly responsive to precipitation because of NO_3-N mobility with drainage water (Meisinger & Ricigliano, 2017; Fraser et al., 2013). Soil texture, weather, and cover crop presence all influence soil moisture which, in turn, impacts N forms and movement (Zhu et al., 2018; Basche & DeLonge, 2019). In this study, three cover crop treatments are compared in two fields with contrasting soil textures, during two years with contrasting weather conditions.

The three cover crop treatments were: 1) no cover crop winter fallow without weed control (NC), a mixture of forage radish (*Raphanus sativus* L.), crimson clover (*Trifolium incarnatum* L.), and cereal rye (*Secale cereale* L.) (3WAY), and a monoculture cover crop of cereal rye (RYE). These were integrated into a corn/soy rotation in two fields with contrasting soil textures, one very sandy, and the other dominated by silt and clay.

Over two years from 2021-2023, we investigated the effects of the three cover crop treatments on,

- Cover crop biomass and tissue N content
- The previous cash crop effect on concentration of NO_3-N in porewater during the 7-month leaching season
- Yields of cash crops corn and soybean, specifically the effect of N rate on corn yield and the cover crop termination time on soybean yield, and cover crop species on yields of both cash crops.

Materials and Methods

Sites and Management

We conducted field experiments at two sites with contrasting soils at the Beltsville Facility of the University of Maryland Central Maryland Research and Education Center (CMREC) (39°00'46.0"N 76°49'35.7"W). One site (called Sandy) was dominantly Downer and Hammonton soils with loamy sand to sandy loam textures throughout the profile, while the other site (called

Silty) was dominated by Christiana and Russet soils with silt loam A horizons over silty clay loam subsoils. Both sites had a history of 35 years of management with no-till farming of corn, soybean, and wheat, with cover crops included whenever possible during the last 20 years of that period.

Plots were arranged in a randomized complete block (RCB) design with split-split plots and four replicates at each of the two sites. The main plots (55m by 27m) comprised of two cash crops (corn or soybean in rotation), planted in 72 cm wide rows. The subplots (55m by 9m) comprised three cover crop treatments, NC, RYE, and 3WAY, and the sub-subplots (18m by 9m) were assigned cover crop termination times EARLY, MID, and LATE, ahead of soybean and N fertilization rates during corn (Table 1-1). Corn was fertilized with three rates, ZERO, MED, and HIGH. The HIGH rate of N was applied according to MDA nutrient management guidelines at of 1 kg N /56.5 kg of anticipated yield (Maryland Nutrient Management Manual I-B1 P1-15, Annapolis, MD). The MED N rate was a more conservative application of about 0.73 kg N/56.5 kg grain. Corn yields at each field in 2021 were lower with MED than with HIGH so both rates were increased in 2022 with a greater increase at the silty field (Table 1-2).

Cover crops were overseeded into standing corn or soybean crops with a Spra Coupe Sprayer (4640, Duluth GA) fitted with an Orbit Air Seeder (62 series 62DS12F, Owatonna, MN) and drop tubes. Seeding rates were 112 kg/ha cereal rye in RYE and 4.5, 17, 84 kg/ha for the radish, crimson clover, and rye, respectively, in 3WAY. Corn and soybean were no-till planted with John Deere MaxEmerge no-till planter (Moline, IL), in 72 cm wide rows with a target of 74,132 and 395,368 viable seeds per hectare, respectively. All plots were managed with no-till techniques, so tillage was not performed during the study. Cover crops were terminated on the dates shown in Table 1-1 by spraying herbicides. Where corn was planted cover crops were also roller crimped.

Table 2-1 Cover crop management at two sites from 2020-2023.

Site	Cover crop planting date into corn	Cover crop planting date into soybean	Cover crops fall sampling date	Cover termination date*		
				EARLY	MID	LATE
Sandy	6/26/20	9/7/20	12/10/20	4/14/21	5/13/21	5/21/21
Silty	6/27/20	9/7/20	12/13/20	4/14/21	5/13/21	5/21/21
Sandy	8/30/21	9/16/21	11/13/21	4/10/22	5/10/22	5/21/22
Silty	9/1/21	9/16/21	11/14/21	4/10/22	5/10/22	5/21/22
Sandy	9/1/22	9/14/22	12/10/22	4/12/23	5/10/23	5/25/23
Silty	9/12/22	9/14/22	12/12/22	4/12/23	5/10/23	5/25/23

** Cover crop termination was by herbicide (In Corn: Glyphosate, 2.34 L/ha, ai 0.96 L/ha, saflufenacil 0.15 L/ha, ai 0.017 L/ha, methylated seed oils 0.710 L/ha. In soybean: Glyphosate, 2.34 L/ha, ai 0.96 L/ha, 2,4-D 1.75 L/ha, ai 0.98 L/ha). Spring cover crop biomass sampling date was 1-2 days before termination.*

Table 2-2 Management of corn and soybean from 2021-2023 at two sites.

Site	Year	Corn Planting	Corn Harvest	Corn side-dress N rates, kg/ha			Soybean Planting	Soybean Harvest
				ZERO	MED	HIGH		
Sandy	2023	May 10th	Sept 26th	0	112	179	May 10th	Oct 10th
Silty	2023	May 9th	Sept 9th	0	123	202	May 9th	Oct 13th
Sandy	2022	May 10th	Sept 19th	0	112	179	May 10th	Oct 25th
Silty	2022	May 12th	Sept 22nd	0	123	202	May 12th	Oct 28th
Sandy	2021	May 4th	Sept 22nd	0	84	168	May 6th	Oct 20th
Silty	2021	May 4th	Sept 20th	0	84	168	May 6th	Oct 19th

Weather

Daily temperature, precipitation, and 30-year averages were determined using Oregon State University software (*PRISM*, 2004) (**Error! Reference source not found.**).

Cumulative growing degree days (GDD_{cuml}) with a mean daily temperature base of 4°C for winter cover crops (Brennan & Boyd, 2012); were calculated as,

$$GDD_{cuml} = \sum(\text{daily mean air temperature} - 4) \qquad \text{(eq. 1)}$$

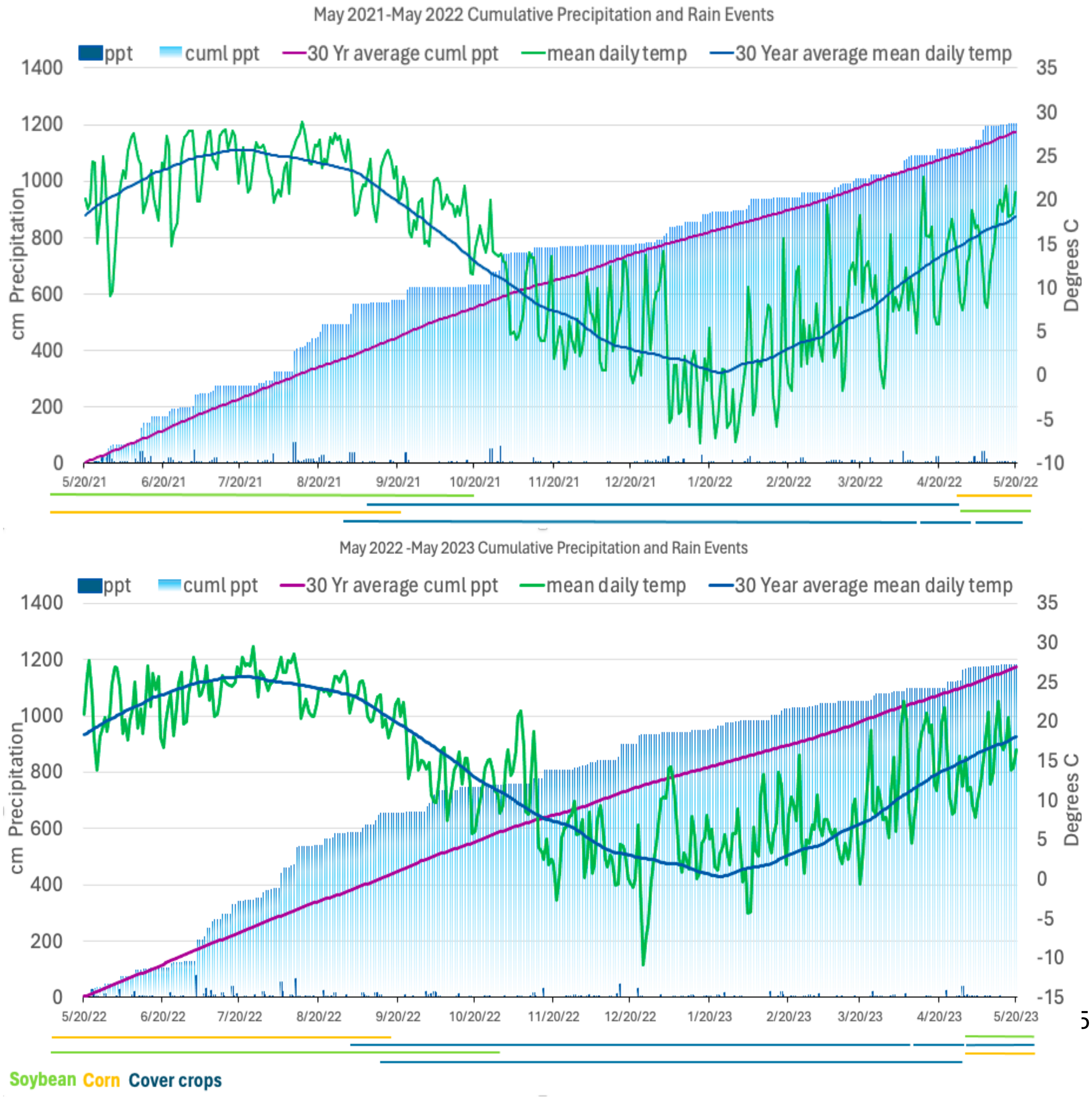


Figure 2-1 Site daily precipitation, cumulative precipitation, mean daily temperature compared to the 30-year average for cumulative precipitation and mean daily temperature and growing season periods for crops and cover crops. Upper panel is for 2021-2022 and lower panel is for 2022-2023. Weather data sourced from PRISM, Oregon State University. Ppt = ppt = precipitation; cuml ppt = cumulative precipitation.

The study took place during two contrasting leaching seasons. The fall of 2021 was far drier than normal and the precipitation that came was uneven with weeks or months without meaningful rain. Cumulative rainfall caught up to the 30-year average for three weeks in the fall but was about 10 cm below the average for the remainder of the sampling period. The mean daily temperatures did not deviate much from the 30-year average, but the fall and first quarter of winter were warmer than normal. The remaining winter temperatures were colder than normal, while the spring was similar to the average. Winter temperatures were cold enough for the radish in the 3WAY to winter-kill. The fall of 2022 displayed different conditions to the previous year. Rain was plentiful and frequent, largely staying in step with the average into the winter. The spring was drier than normal with the cumulative precipitation staying about 5 cm below the average. Mean daily temperatures were well below the average in the early fall. However, temperatures were above average for most of the winter and spring. Winter temperatures were still cold enough for the radish to winter-kill.

Cover Crop Biomass and N Content

Fall cover crop biomass samples were taken in late November- early December before radish had winter-killed but after all the species had stopped growing. Spring cover crop biomass was measured one or two days before termination. Vegetation within a 0.25 m^2 quadrat was clipped 1 cm above the soil surface. This was done at two randomly chosen locations per plot, one near each end of the plot, as a stratified random sample. During the fall biomass collection, both the shoots of radish and the fleshy radish root were collected, the latter is partly growing aboveground and partly pulled up from the soil. The samples, especially the radish roots, were cleaned with tap water to remove soil. Biomass collected at two locations within each sub-subplot was separated by species and consolidated into separate paper bags for each species or component (radish taproots, radish shoots, clover, grass, and weeds) for each plot. The biomass was dried at 60–70˚C to constant weight and the dry weight recorded. Dried tissue for each species of cover crop was weighed and ground (< 1mm) separately. The ground sample was thoroughly mixed and stored in a 20ml vial for total C and N analysis by high-temperature combustion (LECO CN628 analyzer, St. Joseph, MI). Results were expressed as the fraction C and N in the dry tissue. The ratio of C/N was then calculated.

The N content for each species was calculated as:

Dry matter (kg/ha) × N concentration (kg/kg) = kg N/ha (eq. 2)

The N content for each sub-subplot was calculated as

(Grass kg N/ha + clover kg N/ha + weeds kg N/ha + radish kg N/ha + radish taproot kg N /ha) x 0.017 ha = kg N /sub-subplot (eq. 3)

Porewater N Concentrations

During the leaching season from October to May, porewater leachate samples were collected biweekly under 80 kPa tension from ceramic-tipped suction lysimeters and analyzed for nitrate, ammonium and total dissolved N. The suction lysimeters were made of butyrate tubes (22-mm outer diameter) with 100-kPa air entry ceramic tips (Irrometer, Inc. Riverside, CA) and were installed to 90 cm deep at two representative locations in each cover crop x main crop sub-subplot sampled. Lysimeters were installed in the sub-subplot where the previous corn crop received the

HIGH N rate or where the cover crop preceding the previous soybean crop was terminated late. They were installed as soon as possible after cash crop harvest to obtain soil macro-porewater samples from the bottom of the crop root zone beginning in Fall. Before installing each lysimeter, a pilot hole of the same diameter was made using a drop hammer device, and 200 ml of water–subsoil slurry (using soil material from 70-90 cm in the same field) was poured down the hole to ensure good contact between the ceramic tip and the soil. The upper 5–10 cm of soil around each lysimeter was sealed with bentonite to prevent preferential flow. A 75-to-85-kPa vacuum was applied for about 24 hours to collect porewater samples every 2 weeks throughout the cover crop growing season. Porewater samples were pumped into 50 mL centrifuge tubes, kept cold during transport and frozen until analyzed. After thawing, porewater samples were analyzed for pH and electrical conductivity (EC) using a Horiba LAQUAtwin Model PH-11 Compact Water Quality pH Meter and Horiba LAQUAtwin EC-33 Compact Conductivity Meter, then filtered through a 0.45 µm acetate filter membrane, and frozen again until they could be analyzed for NO_3-N by the salicylic acid colorimetric method (Cataldo et al., 1975).

Statistical Analysis

Data were analyzed using R Studio version 1.2.5042, using R packages pacman, lme4, and emmeans. The Shapiro-Wilk test was used to assess normality, and power transformations were applied to satisfy assumptions of normality and homogeneity of variances. If data could not be transformed to a normal distribution, non-parametric tests Kruskal-Wallis rank sum test and pairwise comparisons using Wilcoxon rank sum test with continuity correction with Benjamini-Hochberg p value adjustment method. Soil porewater data was never able to be transformed to a normal distribution. The data was unbalanced because not every lysimeter produced a sample on every sample date. Therefore, only non-parametric tests were used for N concentration data. Crop and cover crop biomass data were analyzed with analysis of variance (ANOVA) and post-hoc analysis with Tukey Honestly Significant Difference (HSD). The effect of Year and Block nested within Field were considered as random effects and fall cash crop and cover crop treatment were considered as fixed effects.

Results and Discussion

Fall Cover Crop N Content

In fall 2021 biomass N was three times as great for 3WAY as for RYE and NC (Figure 2-2) with the difference being driven mostly by greater growth of clover and radish. The total biomass N in the RYE plots were not different than that of weeds in NC. In fall 2022, 3WAY had nearly twice the biomass N content of RYE. The radish biomass in 3WAY was very low in fall 2022, likely due to the unusually cool fall temperatures. The rye in 3WAY made up a larger proportion in the mix than in the warmer fall of 2021. Baraibar et al. (2020) also reported that that cooler temperatures tend to favor cereal cover crop growth while warmer temperatures favored brassica growth. In Argentina, Restovich et al. (2012) reported radish cover crop N contents between 17 - 131 kg N/ha, depending on the year. Using the same mix and growing in the same region, early-planted cover crops studied by Sedghi et al. (2023) averaged 48.8 kg N/ha in the biomass, and cover crops planted after crop harvest averaged 28.2 kg N/ha. Despite being planted early, cover crops in this study had relatively low biomass and N contents in both years, possibly due to the temporal mismatch of temperature and moisture resources. The fall of 2021 had favorable warm temperatures higher than the 30-year average, but little moisture for initial cover crop germination and growth (**Error! Reference source not found.**). Cover crop emergence may have been delayed by lack of moisture during the week following planting. Cover crops seeded into corn received 72 mm precipitation in the week following planting and those seeded into soybean 52 mm. Unlike the previous year, the fall of 2022 had plenty of moisture but unusually low temperatures (Figure 2-1). Sedghi et al. (2023) found that during dry falls cover crop biomass and N accumulation were limited by moisture even when their early sowing provided more GDD. Sedghi et al (2023) reported that during a fall with both good moisture availability and warm temperatures, cover crops accumulated much more N. Since the fall of neither year in the current study provided both favorable moisture and temperature conditions, growth was lower than expected for early planted cover crops.

The N uptake in RYE was notably smaller than by 3WAY , but the N mass lost to leaching from the RYE was not higher than from 3WAY (Figure 2-2, Table 1-3). Meisinger & Ricigliano (2017) also observed that N leachate concentrations under rye were lower than under other cover crop species even though rye had lower apparent N uptake than the other species. We speculate that one possible explanation might be that, since the below-ground rye biomass was not accounted for in either study, rye may have taken up more total above plus belowground biomass N than the other species. However, the more likely explanation is that, since 3WAY contained a N-fixing legume species, crimson clover, some of the N in 3WAY biomass may have originated from the atmospheric rather than from uptake of soil N.

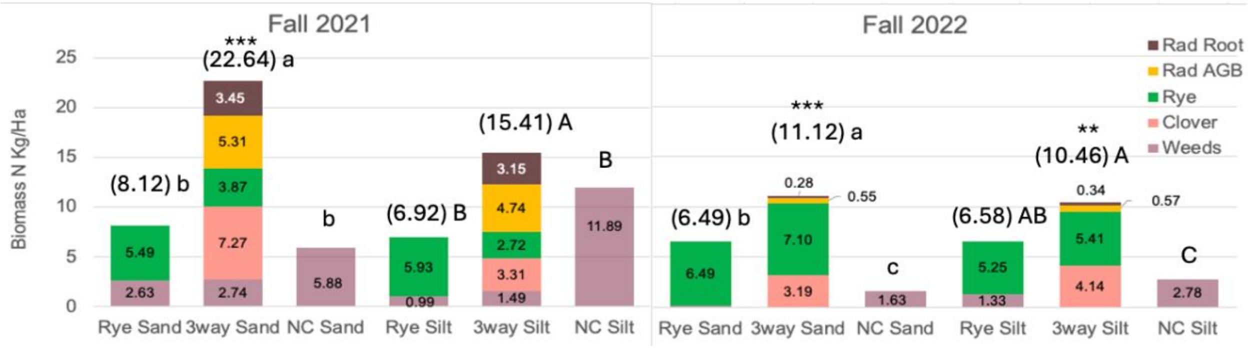


Figure 2-2 Cover crop N content with the average amount produced for each species above-ground biomass (AGB) and the radish root. The total average cover crop N content is displayed in parentheses for each cover crop type at each field in each year. Within each year and site, if followed by a different letter, (lowercase for sandy and uppercase for silty), N contents are significantly different with an ANOVA, Tukey $p<0.0001$ for 2021. For the 2022 comparisons, N contents with a different letter are significantly different with a $p<0.001$ for the silty site and $p<0.0005$ for the sandy site

Growing Degree Days Effect on Cover Crop N Content

C

1.33

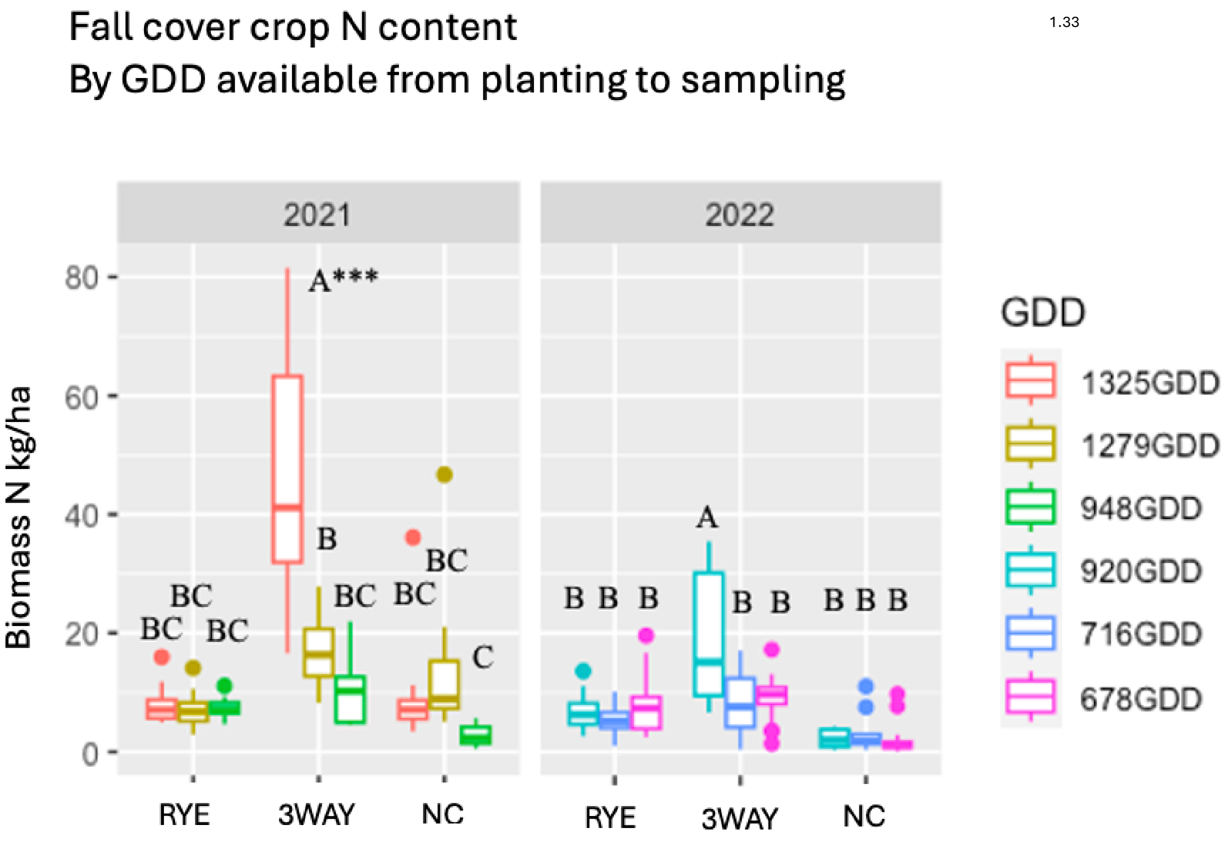


*Figure 2-3 The average of both sites' biomass N in cover crop tissues based on the GDD available that year calculated from planting date until sampling date. Within the same year, boxplots with a different letter are significantly different with an ANOVA, Tukey p value of < 0.05 or <0.0001 "***". The center bar in the boxplots represents the median.*

In 2021 the 3WAY that experienced 1325 GDD contained more N than the RYE that experienced 1325 GDD (Figure 2-3). Also, the N content of RYE was the same regardless of whether it received 1325, 1279, or 948 GDD. The 3WAY that received less then 1325 and RYE that received any level of GDD did not differ from the N content of the weeds in NC. In 2022 there were fewer GDD days available as the highest level of GDD in 2022 is lower than the lowest level of GDD in 2021. Once again, the 3WAY that received the most GDD (920) contained more N than any other cover crop that year, regardless of available GDD, and was the only cover crop to contain significantly more N than the weeds in NC. Due to fall 2022 being unusually cool, more GDD were available between cover crop sowing and biomass measurement in 2021 than in 2022 despite the later biomass measurement in 2022 (December 10th-13th) than in 2021 (November 13th-14th). If, in 2021, the cover crops had been sampled in early December they would have received 45.1 additional GDD. The 3WAY being the only cover crop to respond to the additional GDD suggests that it was sufficiently sensitive to GDD to take up greater amounts of N. Different cover crops received different GDD in the same year because sowing onto soybeans occurred later (at leaf fall) than in corn, and in some cases because precipitation delayed seeding. Cover crops can be intersown earlier into a corn crop because there is sufficient light under a senescing corn canopy. By contrast, in a soybean crop, cover crops cannot be intersown until leaf drop due to the more intense shading under a soybean canopy. The RYE took up the same amount of N regardless of the range of GDD here perhaps because it grew more slowly compared to the radish in 3WAY. Others have observed that brassicas respond well to warm temperatures and grow swiftly, while cool-season grass species tend to grow more slowly and benefit from cooler temperatures (Wahlström et al., 2015; Sedghi et al., 2023).

Effect of Cash Crop on Soil Porewater Nitrate Concentrations

Concentrations of NO_3-N in leachate were higher when the previous main crop was soybean in the 2021-2022 leaching season in the month of November but not in any other month or the following 2022-2023 leaching season (Table 2-3).

Table 2-3 The mean porewater NO_3-N concentration of corn and soybean plots at both fields by month during the 2021-2022 and 2022-2023 leaching seasons. Within a site and cover crop mean concentration with a different lower-case letter are significantly different. The Krusker-Wallis, Wilcox p values for the comparison of NO_3-N concentrations from corn and soybean plots within the same month and year are cited.

** Later soybean harvest delayed lysimeter installation*

	2021-2022			2022-2023		
	Mean NO_3-N mg/L			Mean NO_3-N mg/L		
Month	Corn	Soybean	*p* value	Corn	Soybean	*p* value
October	0.20a	0.66a	*0.286*	1.55	*	*
November	0.08b	1.09a	***0.017***	1.87a	1.07a	*0.492*
December	0.13a	0.04a	*0.766*	1.39a	0.83a	*0.258*
January	0.50a	0.68a	*0.896*	1.80a	0.84a	*0.490*
February	1.14a	1.21a	*0.804*	1.63a	1.33a	*0.691*
March	0.92a	1.07a	*0.990*	0.96a	0.54a	*0.660*
April	0.69a	0.59a	*0.990*	0.90a	0.55a	*0.237*

When the entire leaching season of 2021-2022 is averaged, NO_3-N concentrations were higher under soybean at the sandy field and under the NC and the 3WAY but not the RYE (Table 2-4). Previous main crop had no effect on NO_3-N concetrations at the silty field either leaching season or at the sandy field in the 2022-2023 season. In the same region, Hirsh & Weil (2019) similarly found that there was more residual soil NO_3-N after soybean than corn. In an Illinois study on tile drainage, Gentry et al. (2024) found that cumulative NO^3-N losses could be just as high or higher after a soybean crop as after corn. Also, NO_3-N loss following soybean had little to do with how much fertilizer N was applied to corn the previous year. It is unsurprising that any effect of the previous main crop would be seen most strongly in the fall not long after harvest but with enough time for crop residues and roots to begin to break down and mineralize. The soybean residues and root nodules would have a higher C:N and mineralize N more quickly than corn roots and residues. With the conservative N fertilization of the corn crop there was not very much excess NO_3-N left in the soil to dissolve into leachate so the soybean sourced NO_3-N in soil porewater is higher in comparison but in itself is still very low concentrations of NO_3-N

Table 2-4 The mean NO_3-N concentration by corn and soybean at each field and cover crop during the 2021-2022 and 2022-2023 leaching seasons. Within a site and cover crop mean concentration with a different lower-case letter are significantly different. The Krusker-Wallis, Wilcox p values for the comparison of NO_3-N concentrations from corn and soybean plots within the same site and year are cited.

Site	Cover Type	2021-2022			2022-2023		
		Mean NO_3-N mg/L		*p* value	Mean NO_3-N mg/L		*p* value
		Corn	Soybean		Corn	Soybean	
Sandy	RYE	0.26a	0.41a	*0.386*	0.73a	0.50a	*0.613*
Sandy	3WAY	0.23b	0.48a	***0.039***	1.09a	0.66a	*0.810*
Sandy	NC	0.58b	2.89a	***0.020***	3.01a	2.87a	*0.495*
Sandy	Mean	0.37b	1.46a	***0.0004***	1.61a	1.24a	*0.810*
Silty	RYE	0.22a	0.31a	*0.350*	0.23a	0.31a	*0.586*
Silty	3WAY	0.32a	0.27a	*0.900*	0.49a	0.26a	*0.381*
Silty	NC	1.53a	0.60a	*0.133*	3.20a	1.25a	*0.430*
Silty	Mean	0.66a	0.39a	*0.943*	1.35a	0.59a	*0.912*

Corn Yields

Corn yields at experimental sites were in the range seen in commercial operations around the state with year-year variations driven by weather (Table 2-5). The average corn yield for the state of Maryland in 2021, 2022, and 2023 were 10,357 kg/ha, 10,357 kg/ha, and 10,985 kg/ha (USDA, NASS). Weather in 2021 was variable with cooler temperatures and adequate rain in May-June, very dry in July, and above average temperatures in August-September accompanied by more rain (**Error! Reference source not found.**). The adequate rain during most of this growth period without being overly wet may be the reason yields were better at the silty field, as the sandy field drains more readily. Swift drainage is an advantage during periods of abundant rain but not in periods that are drier. Weather in 2022 was variable with slightly dry conditions in May-June, much more rain in July, but drier conditions returning in August-September. Temperatures were often cooler than normal, especially in May after planting and in August-September. The exceptionally heavy rains in July may be the reason the yields were better at the sandy field as excess moisture drained away. Weather in 2023 between May-September tended to be dry but with rainfall coming in time for tasseling- grain filling so yields were fair at both fields.

Corn yields were affected by cover crops only in some site-years (Table 2-5). In 2021, corn yields at the silty field yielded in the order of 3WAY >RYE>NC but did not differ by cover crop at the sandy field that year. In 2022 yields did not differ by cover crop at the silty field but did at the sandy field in the order of 3WAY>RYE=NC. In 2023, yields once again did not differ by cover crop at the silty field but did at the sandy field in the order of RYE>NC>3WAY. Yields varied by field but just as inconsistently, with the silty field yielding higher in 2021, the sandy in 2022, and yields not differing at all by field in 2023. Cover crops were inconsistently influential to yields but at the sites and years where they were, they often improved corn yields rather than reduced them. Similarly, when cover crop had a significant effect, the highest yielding corn was never the corn grown with NC. This is in direct contradiction of reports that corn yields are reduced with cover crops as reported by Deines et al. (2023) based on satellite analysis of the corn belt during the growing season of 2019-2020.

*Table 2-5 Yields of corn from 2021-2023 at two sites. Yields were measured by hand harvest of two rows 6.1 m in length. In 2022-2023 for the two fertilizer rates, 20 kg/ha of the N was applied at planting and the rest at side dress but in 2021 all the N was applied as a sidedressing at the V5-V7 stage. Yields within the same year with a different letter are significantly different and yields within the same year and site with a different letter are significantly different with an ANOVA, Tukey HSD p value <0.05, *=p<0.01, **= p<0.001, ***=p <0.0001, or noted in parentheses. Yields within the same year and/or site without letters are not significantly different.*

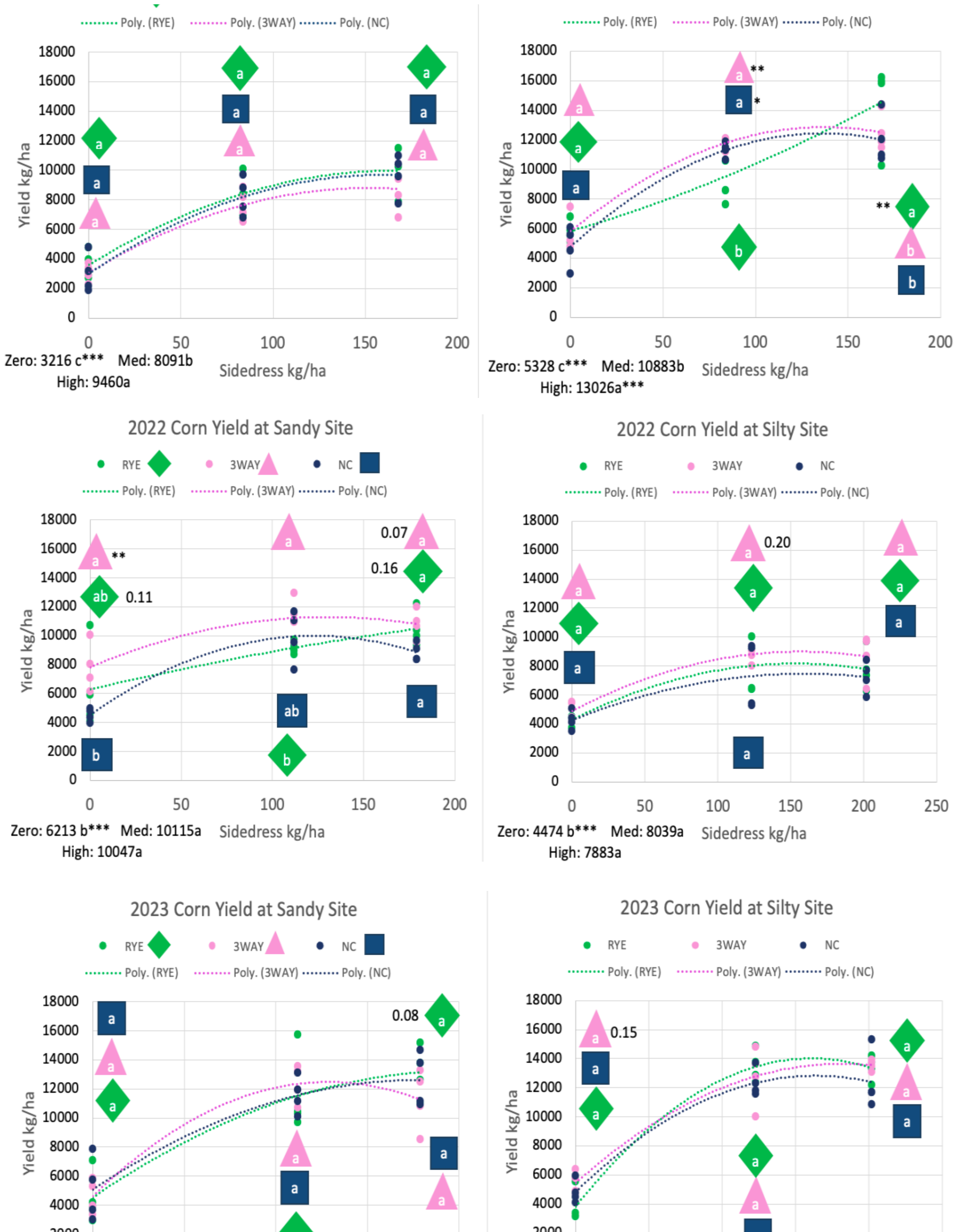
Site	Year	Corn yield (Means of MED and HIGH N rates)			
		kg/ ha			
		Mean of all Cover Treatments	RYE	3WAY	NC

Sandy	2023	12080a *(0.51)*	12338a *(0.92)*	11807a *(0.88)*	12096a
Silty	2023	12972a	13396a *(0.23)*	13166a *(0.40)*	12354a
Sandy	2022	10081a***	9823b *(0.73)*	11014a*	9407b
Silty	2022	7764b	7894b *(0.50)*	8723a*	7233b
Sandy	2021	7543b	6578a *(0.93)*	6512a *(0.64)*	9112a
Silty	2021	11954a***	12044a *(0.89)*	12150a *(0.83)*	11669a

Corn Response to N rate

The HIGH rate of N was applied according to MDA nutrient management guidelines of 1 kg N /56 kg of anticipated yield (Maryland Nutrient Management Manual I-B1 P1-15, Annapolis, MD). The MED N rate was a more conservative application of about 0.73 kg N/56 kg grain. Analysis of the corn yields within each field in 2021 showed a significant yield increase between the MED and HIGH N rates (ANOVA, Tukey HSD p=0.05 at the sandy site and p<0.0001 at the silty site). Because of this response, we increased both the MED and HIGH N rates for 2022 and 2023. Yields in 2022 and 2023 showed no significant difference between the MED and HIGH N rates (p> 0.9 to p= 1.0). This finding demonstrates the value of adaptive management techniques to systematically improve nutrient rates on a field-by-field basis. In this case, the MED rate produced the same yields as the higher state-recommended rate but would save approximately 39-37% on N input costs and lower the risk of N pollution to the environment. One reason the concentration of NO_3-N in porewater measurements during this experiment were so low, even with NC, is likely that the HIGH rate we applied was on the lower end of what is common. Actual application rates on-farm usually exceed state recommendations (Morris et al., 2018), but Maryland nutrient management law prohibits applications exceeding maximum fertilization recommendations, so the HIGH rate is nearer the maximum N application in the context of Maryland agriculture. The MED rate was lower than what most Maryland farmers apply to corn, but only with the lower rates in 2021 was there a significant yield increase between the MED and the HIGH N rate. It should be noted that we sampled porewater only in the corn plots receiving the HIGH N rate (Figure 1-2)

*Figure 2-4 Corn yields at ZERO, MED, and HIGH N rates by cover crop at each field and year 2021-2023. Yields are fitted to a polynomial curve. The mean yield for each N rate is displayed at the bottom left and are compared as ZERO to MED, and MED to HIGH. Yields with a different letter are significantly different with an ANOVA, Tukey HSD p <0.05, **<0.005, ***<0.0001, p values of 0.05<p<0.21 are displayed. Yields within a N rate is compared as RYE to NC and 3WAY to NC*



Soybean Yields

Soybean yields at experimental sites were similar to those of commercial operations around the state with year-year variations driven by weather (Table 2-6). The average soybean yield in the state of Maryland in 2021, 2022, and 2023 were 3161, 2892, and 3564 kg/ha (USDA, NASS). Soybean yields were almost always unaffected by cover crops and their termination times suggesting that cover crops did not negatively affect soybeans through shading or allelopathy though neither of these factors were measured directly. The soybean yields were better under the RYE cover crop at the sandy site in 2021, but no other cover crop effects were observed. Field and year were continuously the most influential variables to yields, not cover crops. The soybean yields were unchanged by planting green (the MID cover crop termination treatment) or by terminating the cover crops 2 weeks after planting soybeans (the LATE cover crop termination treatment). The soybean yields being unaffected by planting green in this study are consistent with what others have observed in no-till soybean studies in the US Mid-Atlantic region (Reed et al., 2019). A recent large-scale analysis using satellite data across the US Mid-west concluded that cover crops reduced soybean yield by 3.5% in years with relatively low precipitation and warm spring temperatures (Deines et al., 2023). The soybean yields in this study contradict the idea that cover crops reduce soybean yields as, even when cover crops were terminated two weeks after soybean planting, soybean yields were not reduced. This was true even when, in 2022, the spring was warmer and drier than the 30-year normal after soybean planting (**Error! Reference source not found.**). Soybean yields were more responsive to year and soil. During the first two years 2021 and 2022 soybeans yielded higher at the sandy field but in 2023 soybeans yielded higher at the silty field. The cumulative rainfall was higher than the 30-year normal during the mid-summers of 2021-2022, so it is possible that sandy field that drained more effectively improved yields compared to the poorly drained silty field. The soybeans at the silty field yielded better in 2023 and the spring and summer of 2023 was drier than the 30-year average with the months of May-August seeing 20% less rain than the 30-year average precipitation for the study location (PRISM, 2004). In a drier year the fast drainage at the sandy field may have no longer been an advantage, leading to higher yields at the silty field which had a higher water holding capacity.

*Table 2-6 Average yields of soybeans from 2021-2023 at two sites. Average yields within the same year with a different letter are significantly different with an ANOVA, Tukey HSD p value of ***=<0.0001, or **=<0.01. Yields within the same year and site with the same letter are not significantly different. The Tukey HSD p values for the comparison of mean soybean yields from EARLY to MID and EARLY to LATE termination times within the same year, field and cover crop are cited in parenthesis*

**Yields were harvested by hand in 2022 and 2023 to measure yields in the cover crop termination treatments. Harvest was done by combine in 2021 and cover crop termination treatment effects on yield not measured.*

Site	Year	Cover type	Mean soybean yield kg/ha *				
			Previous cover crop termination time				All covers all termination times
			EARLY	MID	LATE	Mean	
Sandy	2023	RYE	3529a	3855a *(0.91)*	3296a *(0.96)*	3560a *(0.99)*	3423 b
		3WAY	3838a	2848a *(0.44)*	2720a *(0.35)*	3135a *(0.54)*	
		NC	3793a	3483a *(0.90)*	3448a *(0.92)*	3575a	
		Mean	3720a	3395a *(0.72)*	3155a *(0.38)*	-	
Silty	2023	RYE	5434a	4145a *(0.25)*	4597a *(0.55)*	4725a *(0.84)*	4805 a***
		3WAY	5524a	5197a *(0.91)*	4878a *(0.70)*	5200a *(0.21)*	
		NC	4062a	4715a *(0.70)*	4694a *(0.71)*	4490a	
		Mean	5007a	4685a *(0.73)*	4723a *(0.78)*	-	
Sandy	2022	RYE	4559a	4355a *(0.95)*	4011a *(0.67)*	4308a *(0.90)*	4236 a**
		3WAY	4197a	4540a *(0.85)*	4091a *(0.98)*	4276a *(0.93)*	

		NC	3465a	4229a *(0.46)*	4677a *(0.14)*	4123a	
		Mean	4073a	4375a *(0.76)*	4260a *(0.90)*	-	
Silty	2022	RYE	3564a	4282a *(0.50)*	4007a *(0.77)*	3951a *(0.26)*	3610 b
		3WAY	3561a	3987a *(0.78)*	3199a *(0.84)*	3582a *(0.77)*	
		NC	3577a	3154a *(0.79)*	3156a *(0.79)*	3296a	
		Mean	3567a	3807a *(0.84)*	3454a *(0.96)*	-	
Sandy	2021	RYE	3744 a			-	3628 a***
		3WAY	3672 b **				
		NC	3469 b **				
Silty	2021	RYE	2180			-	2274 b
		3WAY	2344				
		NC	2298				

Conclusion

This study included vastly different management combinations from cover crop presence or absence, cover crop species type, cover crop termination time in soybean, and different N fertilization rates in corn. These management changes had impacts on cover crop biomass and on N leaching but consistently had little to no impact on crop yields. Crop yields were more sensitive to weather changes and site than any management treatment but when yields did differ by management treatment, cover crops often had the better yields. These results demonstrate that cover crops are unlikely to negatively impact yields even when they are managed to have increased presence in the field by early planting and late termination which allows them to provide increased environmental benefits. The 3WAY consistently contained more N and responded more strongly to increased GDD than the single species RYE cover crop. Even in an agricultural system already using sustainable practices like no-till and precise nutrient management, multi-species and single species cover crops still have functions to offer in reducing NO_3-N loss.

Chapter 3 Multi-Species Cover Crop Outperforms Single Species in Biomass and Soil Health

Abstract

Cover cropping is a major tool that agriculture can use to protect soil and water quality and mitigate climate change. Unlike farmland in the world at large, most Maryland cropland has seen little tillage disturbance and some level of cover cropping for decades. With that background, field experiments on two soils with contrasting textures at the Beltsville Facility of Central Maryland Research and Education Center tested the effects of cover crop management enhancements on soil health indicators, and cover crop growth over three years. Two cover crops, sole rye (RYE) and a mixture of forage radish, crimson clover, and rye (3WAY) were compared to a control where cover cropping was ceased (NC). The cash crops were corn and soybean grown in rotation. In spring, cover crops preceding soybean were terminated on three dates from mid-April to mid-May and rye biomass (whether in 3WAY or RYE) doubled with each two-week addition to the growing season. Compared to NC, 3WAY on the sandy soil increased soil permanganate oxidizable carbon (POXC) by 80.5%, total soil carbon by 14.3%, aggregation by 21.8%, and decreased bulk density by 6.4%. RYE increased soil POXC by 47.1% but did not affect other soil health metrics. These results demonstrated the additional benefits of increasing cover crop diversity and biomass production on soil health in an agricultural system already using sustainable practices like no-till, early planted cover crops, and conservative nutrient management.

Introduction

A changing climate with more extreme rainstorms and more severe droughts requires management practices that increase system resilience. Improving, and maintaining improvements, to soil chemical and physical properties can be the key to managing soils for resilience to changing conditions. Resilience includes the ability of soils to drain during wet periods, retain moisture during dry periods, and moderate soil temperature. Overall, soil physical and chemical properties directly and indirectly influence the ecosystem services that soils provide, including absorbing and filtering water, sequestering C, cycling nutrients, and producing high yields of agricultural products. Cover cropping is one of the main tools available for managing the health of agricultural soils. Multi-species cover crops are thought to have greater effects on soil properties than single cover crop species due to the diversity of plant residues (Kallenbach et al., 2016; McDaniel et al., 2016). The diversity of cover crop functional groups including warm and cool season species, grasses, legumes, brassicas can fill different soil ecological needs and contribute to more efficient resource usage (Smith et al., 2014). These specialized benefits may include N fixation, variety in root or canopy structure, and surface residue (Smith et al., 2014). The differences in cover crop functions in a mixture could lead to greater biomass production (Murrell et al., 2017; Smith et al., 2014), which may then differently impact soil ecosystem services (Smith et al., 2014) and eventually change some soil physical and chemical properties (Ruis et al., 2020). Higher biomass is not always achieved by mixes relative to monocultures (Hirsh et al., 2021) but mixes may cycle nutrients more efficiently by lowering N loss in winter while also making captured N available for the following cash crop in spring (Tosti et al., 2014; Hirsh et al., 2021).

The use of cover crops that are capable of vigorous growth and biomass acquisition in the fall can lead to higher biomass in the spring. Compared to a pure cereal rye *(Secale cereale* L.*)* cover crop, brassica species such as forage radish *(Raphanus sativus* L.*)* planted alone or in a mixture with cereal rye, produced more biomass (Dean & Weil, 2009). This combination of deep rooting depth and rapid growth makes radish an optimal biomass-builder and N scavenger in fall. However, when the radish is winter-killed it will release soluble N. This N from the decomposing radish could be lost early in spring in sandy soils (Dean & Weil, 2009; Gieske et al., 2016; Sedghi & Weil, 2022a), making it beneficial to include winter hardy, N scavenging grass species that can utilize N to build biomass in a cover crop mix. Brassica and grass species in a mix maintain N sequestration in fall and spring. The timely release of sequestered N for the following crop is another critical function of cover crops. Legume species, such as crimson clover (*Trifolium incarnatum* L.), are the most consistent at providing N to the following crop (Aronsson et al., 2016; Vyn et al., 1999). Legume species can add additional benefits to a grass-brassica cover crop mix by N fixing in spring and leaving residues with a lower C:N than rye that allows for N mineralization (Ranells & Wagger, 1997). Increasing cover crop duration in the field by earlier planting (Sedghi & Weil, 2022) can improve N capture in the fall and increase amounts of N present in cover crop tissue in the spring. Allowing cover crops to grow longer in the spring (delayed termination) can double or triple the total biomass produced and atmospheric carbon fixed and added to the soil. In Maryland, Sedghi et al. (2023) found that spring cover crop biomass more than doubled when termination was delayed by about 3 weeks. Duiker & Curran (2005) in a study in Pennsylvania found that cereal rye biomass tripled on average when termination was delayed by about three weeks from the early boot to the late boot stage. Also in Pennsylvania, Mirsky et al. (2011) found that cover crop biomass increased approximately 2,000 kg/ha for each 10 day delay in spring termination date between May 1st–June 1st. Delaying cover crop termination can result in higher soil carbon sequestration driven by higher biomass acquisition (Huang et al., 2020; Jian et al., 2020; Blanco-Canqui, 2021; Blanco-Canqui, 2022), and creation of a thicker residue mulch that can conserve soil moisture during hot, dry periods (Alonso-Ayuso et al., 2014). Delaying termination and employing multifunctional cover crop mixes may also retain more N in soils for subsequent crop use (Finney et al., 2016; Blesh, 2018). Weed suppression also increases with increasing cover crop biomass and cover crops terminated too early typically do not reach sufficient levels of biomass (>3,330 kg/ha) to effectively suppress weeds (Mirsky et al., 2011).

However, in the case of winter cereal cover crops, delayed termination may result in higher C/N ratio tissue that immobilizes instead of mineralizes N. Work on delayed termination effects of a cereal rye monoculture cover crop found that rye cover crop terminated within a few days of crop planting usually results in improved N capture but lower N available to the following cash crop due to slow mineralization (Schramski et al., 2021; Krueger et al., 2011). Therefore, farmers delaying termination usually need to apply extra N at planting to compensate for N immobilization by the more mature grass cover crop. Including a brassica and/or a legume species along with a winter cereal may provide the added biomass, carbon, and soil health benefits without the yield-lowering N immobilization (Hirsh et al, 2021). Studies that have compared delayed termination of cover crop mixes to monocultures found that mixes including legumes have similar N capture potential to stands of grass monocultures but also have a lower overall C:N (Lawson et al., 2015; Odhiambo & Bomke, 2001; Koehler-Cole et al., 2020).

The three cover crop treatments were: no cover crop winter fallow without weed control (NC), a mixture of forage radish (*Raphanus sativus* L.), crimson clover (*Trifolium incarnatum* L.), and cereal rye (*Secale cereale* L.) (3WAY), and a monoculture cover crop of cereal rye (RYE). These were integrated into a corn/soy rotation in two fields with contrasting soil textures, one very sandy, and the other dominated by silt and clay.

Over two cover crop-cash crop cycles (2021-2023), we investigated the effects of cover crop termination timing on,

- Cover crop biomass accumulation
- Species proportions and weed suppression
- Yield of the following cash crop

In 2023, after cover crop treatments had been deployed for three years, we investigated the effects of the two cover crop systems on soil health metrics, including

- Soil total organic C and soil permanganate oxidizable carbon (POXC)
- Soil total N
- Amount and wet-stability of macro aggregates
- Bulk density (BD)

Material and Methods

Sites and Management

We conducted field experiments on two contrasting soils (Silt loam/silty clay loam v. Sandy loam/ loamy sand) at Beltsville Facility of the University of Maryland Central Maryland Research and Education Center (CMREC) (39°00'46.0"N 76°49'35.7"W). The experiments compared three cover crop treatments: 1. a mixture of 4.5 kg/ha of forage radish (*Raphanus sativus* L.), 17 kg/ha of crimson clover (*Trifolium incarnatum* L.), and 84 kg/ha of cereal rye (*Secale cereale* L.) (3WAY), 2. a monoculture of 112 kg/ha cereal rye (RYE), and 3. an un-weeded no-cover crop control (NC). These were integrated into a corn/soy rotation in two fields with contrasting soil textures, a sandy loam, and a silty clay loam. Cover crop treatments were subjected to three termination timings in the spring: two weeks before main crop planting (EARLY), at main crop planting (MID), and two weeks after main crop planting (LATE). A randomized complete block experimental design was used with split-split plots and four replicates at each of the two sites. Cash crop (corn-soybean or soybean-corn rotation) was assigned to main plots (55m by 27m), planted in 72 cm wide rows, cover crop treatments were assigned to subplots (55m by 9m) and sub-subplots were assigned cover crop termination timing treatments ahead of soybeans, or corn N fertilization rates ahead of corn (18m by 9m). Any sampling of biomass or soil was taken from at least 1m from a plot edge. Cover crops were planted early by overseeding into standing corn (*Zea. mays)* or soybean (*Glycine max*) canopy using a Spra Coupe Sprayer (4640, Duluth GA) fitted with an Orbit Air Seeder (62 series 62DS12F, Owatonna, MN) and drop tubes. Cover crop seeding rates were 112 kg/ha for the RYE cover crop and 4.5, 17, and 84 kg/ha for the radish, crimson clover, and rye for the 3WAY mix cover crop. Corn and soybean were no-till planted with John Deere MaxEmerge no-till planter (Moline, IL), in 72 cm wide rows with a target of 30,000 and 160,000 viable seeds per hectare, respectively. Cover crop termination was carried out with herbicides (Table 3-2). Cover crops were also roller crimped ahead of corn. Corn nitrogen fertilization rates were none (ZERO), medium (MED) (0.73 kg N / 56 kg expected grain yield), and high (HIGH) (1 kg N/ 56 kg expected grain yield) and varied slightly among years and soils as shown in Table 3-3. All plant and soil samples were collected at least 1 m from any plot edge.

Composite soil samples (1.85 cm diameter cores from 0 to 15 cm depth) were taken in June of 2023 from random representative locations within each plot at least 1 m from the edge. The soils were air-dried, sieved to <2mm and analyzed for OM (loss on ignition), pH (2:1 in water), soil texture by feel, and Mehlich 3 extractable nutrient contents (Waypoint Analytical lab, Richmond, Virginia) (Table 3-1).

Table 3-1 The means of soil chemical properties from 0-15 cm in depth at each site averaged across reps as sampled in June 2023. The Mehlich3 soil test extractant was used for all nutrient values and estimated CEC. Soils *were sampled as described above and a composite sample made by combining an equal mass (to 0.0001g) from each sub-sub plot to produce a sample for each main crop, cover crop in each replicate at each field. No significant differences (p<0.05) were found except between sites and soil test results were averaged and presented by site.*

Characteristic	Sandy Site	Silty Site
A-Horizon	Loamy sand to sandy loam	Silt loam
B-Horizon	Sandy loam	Silty clay loam to silty clay
Organic matter g/kg	1.42	2.37
Estimate CEC cmolc/kg	3.03	5.26
pH	5.99	6.03
Phosphorus ppm	64.33	17.00
Potassium ppm	86.33	42.83
Calcium	347.5	655.33
Magnesium	64.75	123.75
Sulfur	10.5	10.0
Manganese	32.08	52.58
Zinc	1.38	1.76
Iron	109.67	129.83
Copper	0.53	1.14
Boron	0.13	0.28

Table 3-2 Cover crop management at two sites from 2020-2023.

Site	Cover crop planting date into corn	Cover crop planting date into soybean	Cover crops fall sampling date	Cover termination date*		
				EARLY	MID	LATE
Sandy	6/26/20	9/7/20	12/10/20	4/14/21	5/13/21	5/21/21
Silty	6/27/20	9/7/20	12/13/20	4/14/21	5/13/21	5/21/21

Sandy	8/30/21	9/16/21	11/13/21	4/10/22	5/10/22	5/21/22
Silty	9/1/21	9/16/21	11/14/21	4/10/22	5/10/22	5/21/22
Sandy	9/1/22	9/14/22	12/10/22	4/12/23	5/10/23	5/25/23
Silty	9/12/22	9/14/22	12/12/22	4/12/23	5/10/23	5/25/23

** Cover crop termination was by herbicide (In Corn: Glyphosate, 2.34 L/ha, ai 0.96 L/ha, saflufenacil 0.15 L/ha, ai 0.017 L/ha, methylated seed oils 0.710 L/ha. In soybean: Glyphosate, 2.34 L/ha, ai 0.96 L/ha, 2,4-D 1.75 L/ha, ai 0.98 L/ha). Where corn was planted cover crops were also roller crimped. Spring cover crop biomass sampling date was 1-2 days before termination.*

Table 3-3 Management of corn and soybean from 2021-2023 at two sites.

Site	Year	Corn Planting	Corn Harvest	Corn side-dress N rates, kg/ha			Soybean Planting	Soybean Harvest
				ZERO	MED	HIGH		
Sandy	2023	May 10th	Sept 26th	0	112	179	May 10th	Oct 10th
Silty	2023	May 9th	Sept 9th	0	123	202	May 9th	Oct 13th
Sandy	2022	May 10th	Sept 19th	0	112	179	May 10th	Oct 25th
Silty	2022	May 12th	Sept 22nd	0	123	202	May 12th	Oct 28th
Sandy	2021	May 4th	Sept 22nd	0	84	168	May 6th	Oct 20th
Silty	2021	May 4th	Sept 20th	0	84	168	May 6th	Oct 19th

Growing Degree Day Calculation

Cumulative Growing Degree Days (GDD, °Cd) during the cover crop growing period were calculated as:

$$GDD = \sum (T_m - T_b) \qquad \text{(eq. 1)}$$

Where T_m is the average daily temperature and T_b (4 °C) is the base temperature (McMaster & Wilhelm, 1997; Moot et al., 2000; Brennan & Boyd, 2012). The GDD contribution was set to 0 when $T_m < T_b$. The cover crop growing period for spring biomass measurements was considered as the period from December 1st until sampling of above-ground biomass of the cover crop just before spring termination.

Pre-sidedress Nitrate Test

In 2022 and 2023, when corn was at the V-4 to V-6 stage of growth (Hanway, 1963), a total of six soil cores per cover crop subplot were collected by taking two cores in each of the three N rate sub-subplots (1.84 cm diameter × 30 cm deep separated at 0-15 cm and 15-30 cm) for a pre-sidedress nitrate test (PSNT) (Meisinger et al., 1992). The six cores were combined into one composite sample per cover crop treatment plot, stored in plastic bags on ice while transported to the lab, then rapidly air-dried under fans at room temperature. Air dried soils were sieved to pass <2 mm, and 2.00 g of dry soil added to 20.0 mL of 0.5 *M* K_2SO_4 in a 50 mL centrifuge tube and shaken horizontally (200 rpm for 30 min). The supernatant was filtered through a 0.25 µm acetate membrane. The filtrate was analyzed for NO_3-N using the salicylic acid method (Cataldo et al., 1975).

Surface Soil Sampling for Soil Health Indicators

Soil was sampled from sub-subplots for HIGH and ZERO N rates in corn and EARLY and LATE cover crop termination times in soybean. A stainless-steel cylinder (7 cm tall, 3.4 cm radius) was driven vertically into the soil to about 6 cm depth. Then a second empty cylinder was placed on the first cylinder and used to drive the first cylinder to a depth of exactly 7 cm without compacting the surface of the contained soil. The cylinder was then carefully excavated, and the soil was sliced flush with each end to collect a known volume of undisturbed soil. The volume of the core collected in the cylinder was 254.3 cm^3. Four such cores were collected from each sub-subplot from the middle between crop rows that received no wheel traffic from farm equipment. Each of the four cores was labeled separately and stored at 4°C until process within 48 hours. Total sample mass was recorded and a weighed soil subsample was weighed and dried at 105°C for 24 hours to determine the gravimetric water content which was used to calculate the oven dry mass of the whole core sample so that soil bulk density could be determined using the sample volume and total sample oven dry mass (Blake & Hartge, 1986). The remaining soil sample was gently crushed and air-dried for three days at room temperature. The air-dried soil was sieved through a nest of a 6 mm and a 2 mm sieve to record the mass of the sample dry-sieved macro-aggregates (2-6 mm diameter). A small sub-sample of these aggregates was reserved for wet-aggregate stability testing. Another sub-sample was sieved to <2 mm for chemical analysis.

Permanganate-oxidizable Organic Carbon

Permanganate oxidizable C (POXC) was determined using the method of Weil et al. (2003) as modified in S. T. Lucas & Weil (2012) which uses 2.5 g of soil instead of 5 g.

To analyze POXC, 2.5 g of soil was reacted with 20 ml of 0.02 *M* $KMnO_4$ solution and 0.1 *M* $CaCl_2$ in a 50-ml conical centrifuge tube. Samples were shaken horizontally for 2 min on a reciprocating shaker at 180 rpm and settled for 10 min. A 0.5-ml aliquot of the supernatant was pipetted into 49.5 ml of deionized water in another conical centrifuge tube, capped, and mixed by handshaking. Colorimetry was performed using a wavelength of 550 nm on a spectrophotometer. To determine postreaction sample $KMnO_4$ concentrations, absorbance values were compared with a standard curve having known $KMnO_4$ concentrations that ranged from 0.005 to 0.02 mol L^{-1}. Using absorbance, POXC was calculated as:

$$\mathrm{POXC(mg/kg)} = [0.02\mathrm{mol/L} - (a + bz)] \times (9{,}000\mathrm{mgC/mol}) \times \left(\frac{0.02\mathrm{Lsolution}}{0.0025\mathrm{kgsoil}}\right) \qquad \text{(eq. 2)}$$

Where the initial concentration of the $KMnO_4$ is 0.02 mol/L, *a* and *b* are the intercept and slope of the standard curve, *z* is the sample absorbance at 550 nm, 9,000 mg C /mol represents the assumed amount of C oxidized per mole of MnO_4, and 0.0025 kg soil is the amount of sample used in the reaction.

Wet Aggregate Stability

The percentage of water stable aggregates (WSA) was determined on 2-6-mm diameter aggregates by a modification of the Kemper & Rosenau (1986) method as described in Gruver & Weil (1998). A 10 g sample of aggregates (sized 2-6 mm) was placed on a 75 mm diameter sieve with 0.5 mm mesh (BelArt, Inc) that was submerged in a pre-weighed 500 ml cup containing 100 ml of deionized water (C1). The cup was shaken for 2 minutes on an orbital shaker at 100 rpm with an orbit diameter of 1.75 cm. After 2 minutes of orbital shaking, the sieve was removed and submerged in a second pre-weighed cup (C2) containing 100 ml of water. All remaining aggregates were manually disrupted so that all soil particles and aggregates < 0.5 mm, were transferred into the second cup. Both cups were placed in a forced air-drying oven and dried at 80 °C to constant weight (±0.001 g). The percent WSA was calculated as:

$$\%\ \mathrm{Stable} = \frac{100 * (c2)}{(c1 + c2)} \qquad \text{(eq. 3)}$$

where c1 is the combined mass of soil that passed through the 0.73-mm sieve into C1 and the sand fragments that remained on the sieve after forcing aggregates through the mesh into the second beaker, and c2 is the mass of stable aggregates collected in C2.

Statistical Analysis

Data were analyzed using R Studio version 1.2.5042 with R packages lme4, emmeans, and pacman. The Shapiro-Wilk test was used to assess normality, and power transformations were applied to satisfy assumptions of normality and homogeneity of variances. Crop yields, cover crop biomass, and soil health data were analyzed with analysis of variance (ANOVA) and post-hoc analysis with Tukey Honestly Significant Difference (HSD). The effect of year, and block nested within the field were considered as random effects and cash crop, cover crop type, and termination treatment (present only within the soybean crop level in split plots) were considered as fixed effects.

Results and Discussion

Spring Cover Crop Biomass

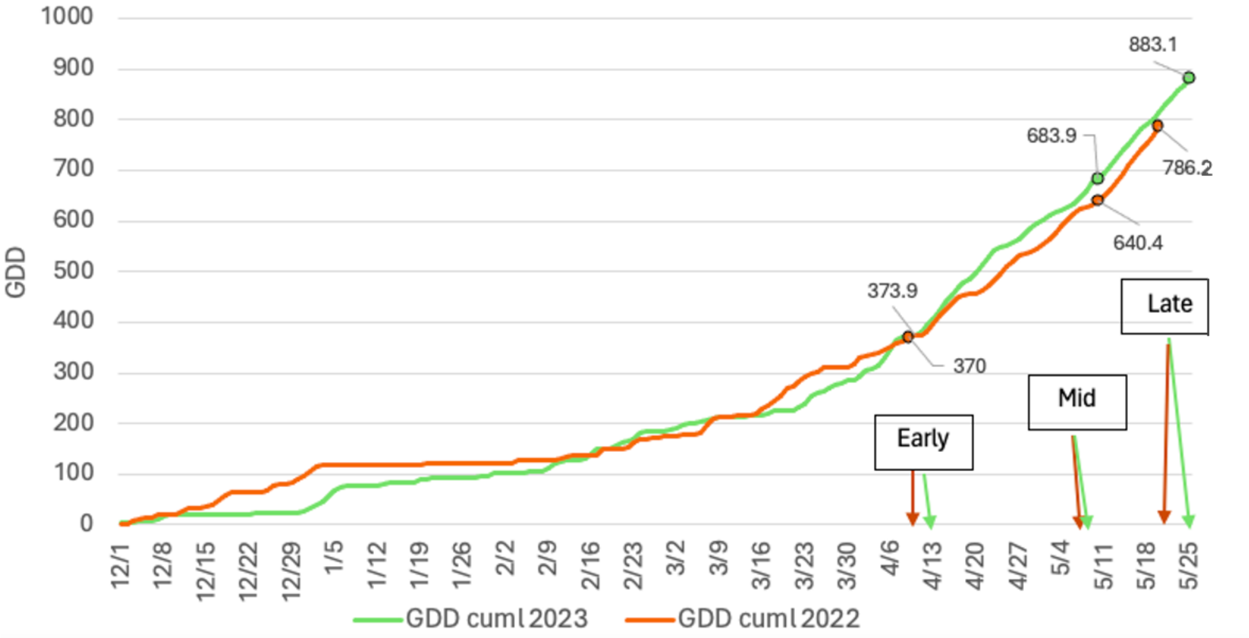


Figure 3-1 The cumulative GDD from Dec 1st until the cover crop termination times in 2022 and 2023 averaged across sites. The GDD for each year were very similar with the rate of GDD increasing sharply around the end of March-early April. The GDD total at each termination time is labeled with a black circle for each year and the GDD level for the early termination time overlap for the two years.

As expected, the MID received more GDD than EARLY with 270.4 more in 2022 and 310.0 more in 2023 (Figure 3-1). On average the 3WAY grew 1,146.63 kg/ha more biomass between Early and MID, RYE grew 1,091.42 kg/ha more (Table 3-4). LATE received more GDD than MID with 145.8 more in 2022 and 199.2 more in 2023 (Figure 3-1). From MID to LATE 3WAY grew 3206.28 kg/ha more and the RYE grew 3,317.59 kg/ha more. The weeds growing in the NC increased only marginally from EARLY to MID to LATE and not to a statistically significant extent.

*Table 3-4 Cover crop biomass at EARLY, MID, and LATE termination times and the rate of growth of biomass per GDD received at that time. Means across two years and two sites. Within a cover crop type biomass with a different letter are significantly different than the next biomass in the series with an ANOVA, Tukey p<0.05, *= p<0.005, **=p<0.001, and ***=p<0.0001*

Cover crop	**EARLY**		**MID**		**LATE**	
	kg/ha	kg gain per GDD since Dec. 1	kg/ha	kg gain per GDD since EARLY	kg/ha	kg gain per GDD since MID
3WAY	3117.62 c	8.38	4264.25 b*	3.98	7470.53 b**	21.2
RYE	752.88 c	2.02	1844.3 b***	3.78	5161.89 a***	21.9
NC	634.84 a	1.71	903.98 a	0.93	1075.95 a	1.10

The rate of cover crop growth per GDD differed between the cover types and terminations. The 3WAY had on average 8.38, 3.98, 21.2 kg/ha/GDD for EARLY, MID, and LATE, respectively, while the RYE grew at 2.02, 3.78, 21.9 kg/ha/GDD (Table 3-4). At EARLY the RYE could not match the same rate of growth as the 3WAY but at MID and LATE they grew at similar rates. For both cover crops it wasn't until the latest termination time that growth rate meaningfully increased with it going down slightly from EARLY to MID. The NC rate of growth changed very little over time from 1.71, 0.93, and 1.1 kg/ha/GDD from EARLY, MID, and LATE, respectively.

When the termination treatments are averaged, the rate of cover crop growth with GDD since December 1st was 7.96 kg/GDD/day for 3WAY and 3.67 kg/GDD/day for RYE. The cover crop rate of total biomass growth was similar to those that others have observed. Working with the same species mix in Maryland, Sedghi et al. (2023) found when radish winter-killed the cover crops grew at a rate of 6.4 to 6.9 kg/ha/GDD. However, if the radish did not winter-kill the spring rate of growth doubled. Also in Maryland using a hairy vetch and cereal rye mix cover crop, Clark et al. (1997) measured an average rate of growth of 8 kg/ha/GDD which is even more similar to the rate observed in the 3WAY but higher than RYE in this study.

Biomass Species Proportions

*Table 3-5 Cover crop biomass by species averaged across two sites and two years. Total mean biomass for each cover crop treatment at EARLY, MID, and LATE termination times. Within each cover type, species biomass at EARLY, MID, and LATE with a different letter are significantly different. Within a termination time, total biomass values followed by a different letter are significantly different. ANOVA, Tukey p<0.05, *= p<0.005, **=p<0.001, and ***=p<0.0001*

Sampling time	Cover Crop	**Rye kg/ha**	**Clover kg/ha**	**Weeds kg/ha**	Total cover crop biomass, kg/ha
EARLY	**3WAY**	562 c	2416 a	137 b	3117 a***
	RYE	589 c	-	164 b	753 b
	NC	-	-	635 b	635 b
MID	**3WAY**	1111 b***	2830 a	324 ab	4264 a ***
	RYE	1641 b***	-	203 ab	1844 b**
	NC	-	-	904 a*	904 c
LATE	**3WAY**	2713 a*	4263 a	494 a*	7471 a**

	RYE	4639 a*	-	523 a*	5162 b**
	NC	-	-	1076 a*	1076 c

Spring termination timing significantly affected the total amount of cover crop biomass and the species proportions (Table 3-5). The 3WAY mix consistently had the highest total biomass regardless of termination timing. The radish winter-killed in both years of the study, so the 3WAY consisted of crimson clover and rye in the spring. The clover biomass was high even at the EARLY in mid-April when the rye cover crop was small enough that its biomass weight was not different than the weeds in NC (Figure 3-2). The clover biomass increased slightly at each termination time but not to a statistically significant extent. Though RYE biomass was small at the early termination, it more than doubled by MID and double again by LATE. The weed biomass in NC did not change between EARLY and LATE and was always smaller than 3WAY and smaller than RYE at MID and LATE. In 3WAY, the clover always made up the largest fraction of the total biomass, but to a lesser degree as the spring advanced. The rye biomass increased over the spring while the clover biomass did not increase to a statistically significant extent (Figure 3-2). For EARLY, clover made up 78% of the total 3WAY biomass while rye made up only 18%, but for LATE, clover made up 58% and rye 36%. This is likely because the clover had already accumulated much more biomass than the rye even at EARLY. As in the current study, Alonso-Ayuso et al. (2014) found that delaying termination time increased cover crop biomass for barley but not for vetch. Weed biomass in RYE and 3WAY was equal to NC at each termination time, suggesting that 3WAY and RYE both did not suppress weeds even when total cover crop biomass differed between them. Other studies of cover crop mixes reported similar results. Though Smith et al. (2014) found higher biomass in a multispecies cover crops compared to monoculture, their mix did not provide superior weed suppresion relative to the most effective monocultures. The lack of any weed suppression by the cover crops in this study despite good levels of cover crop biomass is likely because the weed pressure was low overall. Weed biomass was always less under cover crops compared to NC by between 52-78%, but since weed biomass was low overall this did not result in a statistically significant difference.

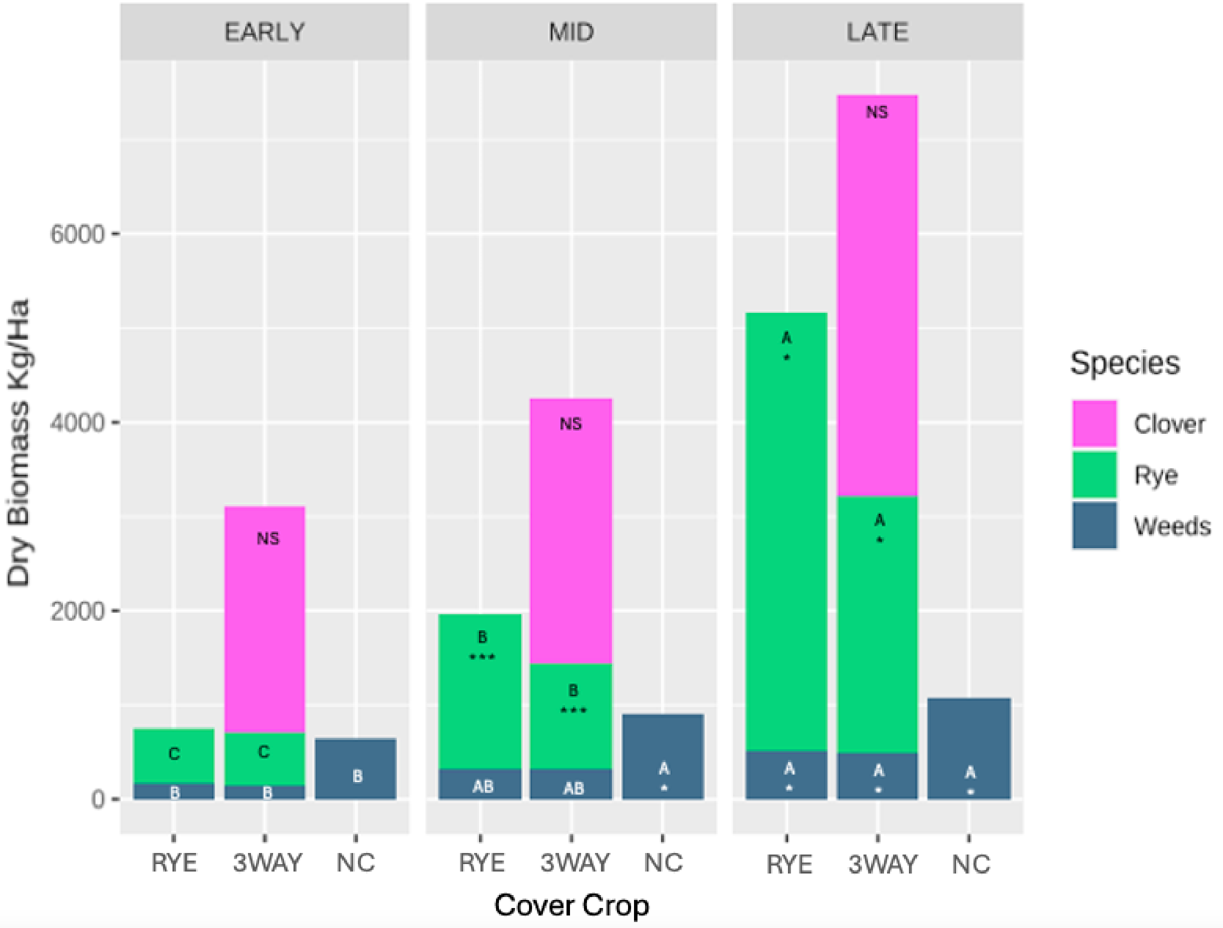


Figure 3-2 Cover crop dry matter for each species within each cover type are compared at EARLY to MID, and MID to LATE. Within each cover type, species biomass at EARLY, MID, and LATE with a different capital letter are significantly different with an ANOVA Tukey p<0.05, p<0.0005*** Means across four site-years,*

Soil Health Properties

Table 3-6 The mean soil health properties at two sites by cover crop. Samples to 7cm depth were collected in June of 2023, one month after planting corn/soybeans and cover crop termination. Effects of soil and cover crop on means for a soil health indicator for cover crops at the same site followed by a different letter are significantly different with an ANOVA, Tukey HSD $p<0.05$, **=$p<0.005$, ***=$p<0.0001$. Capital letters are used for the silty site and lower-case for the sandy site.

Cover crop x Site	Soil H_2O g/g at the time of sampling	BD	Macro-aggregate percent by weight	Aggregate wet stability percent	POXC mg/kg	C g/kg	N g/kg
RYE Sand	0.0938a	1.39 b	16.87 b	97.21a	223.98 a***	0.99 b	0.094a
NC Sand	0.0914a	1.40 b	19.97 ab	95.97a	152.24 b	0.98 b	0.090a
3WAY Sand	0.0915a	1.31 a	24.33 a**	97.20a	274.86 a***	1.12 a	0.105a
RYE Silt	0.1706A	1.31A	65.99A	98.44A	389.73A	1.49 B	0.156A
NC Silt	0.1750A	1.30A	64.68A	98.31A	361.82A	1.42 B	0.145A
3WAY Silt	0.1782A	1.28A	63.40A	98.43A	412.70A	1.55 A	0.158A

Cover Crop Effect on PSNT and Total N

The cover crop had no effect on a pre-sidedress nitrate test (PSNT) taken in June of 2023 despite the crimson clover biomass that made up the largest component of the mulch in the 3WAY plots. Water extractable soil nitrate concentration was consistently low from 0.98-2.58 mg/kg soil with an average of 1.80 mg/kg over both fields (Table 3-6). This is far below the level of NO_3-N required to significantly reduce fertilizer applications (20 to 25 mg/kg) (Meisinger et al., 1992). Hirsh et al. (2021) also did not find a difference in June PSNT soil NO_3-N level between no-cover and a brassica-cereal-legume mix cover crop on mid-Atlantic farms. Also in the mid-Atlantic, Sedghi et al. (2023) did not find a cover crop effect on PSNT taken in June over three years even with high amounts of crimson clover biomass in an early planted and late terminated cover crop. The absence of a cover crop effect on the PSNT is common on no-till soils due to the slower speed of decomposition compared to the rapid mineralization observed when residues are plowed. In a silt loam in Kentucky, Utomo et al. (1990) found in the 0-7.5 cm depth after 5 weeks, a tilled vetch cover crop had mineralized an additional 105 mg N/kg of soil when compared to an un-tilled vetch chemically terminated at the same time. Residues left on the surface break down more slowly and the rate at which they break down is often weather dependent requiring warmer temperatures and more moisture.

Total soil N was also measured via combustion analysis, but in the 0-7 cm depth instead of the 0-15 cm for the PSNT. While there were no statistically significant differences by cover crop, the 3WAY consistently had the highest N percent, NC the lowest, with RYE intermediate at both fields. The silty site had total N percents ranging from 0.145%-0.158% and the sandy site 0.09%-0.105%. Others also did not find statistical differences in soil N% between mixed species cover crops and no-cover (Sharma et al., 2018; Acuña & Villamil, 2014), and individual cover crop species and no-cover (Acuña & Villamil, 2014).

Cover Crop Effect on Soil Organic C and POXC

At the sandy site the soil C increased from 0.98 g/kg C in NC to 1.12 g/kg C in 3WAY, an increase of 0.14 g/kg ($p<0.05$) (Table 3-6) . At the silty site the soil C increased from 1.42 g C /kg soil in NC to 1.55 g C /kg in 3WAY, an increase of 0.92 g/kg ($p<0.05$). Both sites followed the pattern of 3WAY increasing the soil C% the most in comparison to the NC, with RYE increasing it to a lesser degree and never to a statistically significant extent.

Cover crop biomass has been reported to strongly correlate with soil carbon (Ruis et al., 2020) so it is expected that the higher biomass producing cover crop would have higher soil C. The multi-species 3WAY cover crop consistently produced higher biomass compared to the RYE and also had higher average soil carbon after three years of cover cropping. In a review of U.S. studies, Blanco-Canqui (2022) found that cover crop mixes typically do not outperform monocultures in increasing soil C content due to the cover crops having similar levels of biomass. In this study, the mix cover crop consistently had higher biomass levels which translated to a higher level of C stored into the soil. Average soil C increase was 0.41 Mg/ha per year compared to no-cover (Blanco-Canqui, 2022) which is similar to soil C increases of 0.426 and 0.397 Mg of C /Ha per year with the 3WAY at the sandy and silty field, respectively.

In Ohio fields with long histories of no-till and cover cropping, Stavi et al. (2012) found that a mix cover crop of brassica and legume increased soil C by 22% compared to a single species brassica cover crop over ten years but did not compare these with a no-cover control. In Nebraska, Sharma et al. (2018) found that a mixed species cover crop increased soil C by 35% compared to no-cover over seventeen years, while in Argentina Duval et al. (2016) found a mix cover crop increased C by 26% compared to no-cover over just six years. These studies all sampled 0-5 cm soil, while in the present study 0-7cm was sampled. Heterogeneous changes in soil C stratified at shallower depths may be the reason the effect of cover crops at the silty site was too small to be statistically significant. Blanco-Canqui et al. (2014) reported that after three years, a rye cover crop increased soil C in the 0–2.5 cm of the soil, but not in the 2.5–5-cm depth. Soil changes in no-till systems tend to occur first and most markedly at shallow depths. If this stratification was occurring, we may have observed a more pronounced increase in the soil C at both fields. In a review of no-till U.S. studies Blanco-Canqui (2021) cover crops were more likely to increase soil C in sandy soils that have a lower starting soil C level compared to finer textured soils that naturally include more C. In a global meta-analysis Jian et al. (2020) showed that cover crop mixtures result in greater gains in soil C compared to monocultures; in particular, including legumes caused greater soil C increases than using grass species.

POXC has been shown to be an early indicator of soil health improvements and is correlated to changes in soil C (S. Lucas & Weil, 2021). Since reductants other than organic C may be involved in the reaction to a small degree, technically it is more accurate to refer to this test as an indicator of soil permanganate reducing power. For the sandy soil, both RYE and 3WAY had greater POXC than NC ($p<0.05$), while for the silty soil the pattern was similar, but with less statistical certainty ($p=0.18$). While RYE had no measurable effect on soil C concentration, the POXC results suggest that the RYE is still building C, just more slowly than 3WAY, so the effect was not statistically significant in the timeframe of two years of cover cropping treatments. At a nearby site with very similar soil, Lucas & Weil (2021) did not find an effect on POXC (0-7.5cm) after two years of a rye cover crop – corn grain system, while Wang et al. (2017) in another study nearby, did find that a single year of a pure radish cover crop increased POXC in both the surface and subsurface soil compared to no-cover crop in a corn silage system.

Bulk Density and Macro-aggregates

Cover crop effects on soil physical characteristics were most evident for 3WAY at the sandy field, where bulk density was 1.40 g/cm^3 in NC and 1.31 g/cm^3 in 3WAY ($p=0.05$), a difference of 6.4%. On the silty soil the bulk density was 1.30 and 1.28, respectively for NC and 3WAY, not statistically significant ($p=0.52$). In Nebraska, Ruis et al. (2020) found a cover crop mix of legumes, winter cereal and a brassica, did lower BD in a silt loam soil compared to no-cover crop by 7% in the 0–5-cm depth after four years. Despite the differences in texture, this is very similar to the 6.43%, decrease in BD seen at the sandy field (sandy loam) with the 3WAY mixed species cover crop. Why the silt loam soil in this study did not display a lower BD may be because there was no discernable increase in soil C from cover crops at the silty field. In a review Blanco-Canqui & Ruis (2020) found that cover crops reduced bulk density in 31% of 51 studies and improved wet aggregate stability in 50% of 29 studies. However, their correlation analysis for 27 study locations showed that bulk density decreased as SOC concentration increased ($r = -.55$; $p = <.001$; $n = 79$) in the upper 30-cm depth. Soil organic C possibly reduced bulk density by promoting both soil aggregation and biological activity. The increase in soil C seen in the 3WAY cover crop is likely paired with the lowering of the BD.

At the sandy field, the mass proportion of macro-aggregates ($\geq$2mm), from dry sieving, was higher under the 3WAY than in RYE, but not different from NC. The mass proportion of macro-aggregates was the same for all cover crop treatments at the silty field. It was unexpected for the RYE to result in the lowest macro-aggregates as increasing residue input to a cropping system is expected to improve soil aggregation and lead to greater proportions of larger aggregates (Hammerbeck et al., 2012; Stetson et al., 2012). The RYE always produced more biomass than the NC, as long as it was terminated no sooner than May 1st, though never in such great amounts as the 3WAY.

The wet- stability of the dry sieved soil aggregates was unchanged by cover crop treatments at either field, likely because they were on average highly stable whether they were in no-cover or cover crops. Both sites have been under no-till with cover crops for decades and it is likely the reason the macro-aggregates wet stability was between 96-98% for all cover crop treatments and soil textures.

Overall Soil Differences

Figure 3-3 The average percent difference between seven soil measurements under NC and those under the two cover crops with the sandy site on the left and the silty on the right. Data labels for each percent change for the RYE cover crop are on the inner ring and the 3WAY on the outer ring. As the percentages represent the cover cropped soils difference from the soil in NC, so the zero on the axis is the NC.

When RYE and 3WAY are compared to NC, The average relative difference in the seven soil metrics studied show that the cover crop effects were greater for the sandy soil and that 3WAY had greater effects than RYE (Figure 3-3). For the sandy soil, the cover crops enhanced soil health parameters by as much as + 81% (POXC mg/kg) to 0% (soil H_2O) for the 3WAY, and by +47% (POXC) to 1% (macro-aggregates, aggregate stability) for the RYE. In contrast, for the silty soil, the two cover crops had much less influence, with the greatest effect being 14% higher POXC with 3WAY. The 3WAY had a statistically significant effect on the sandy site soil C ($p<0.05$), POXC ($p<0.0001$), BD ($p<0.005$), while the RYE had an effect on the POXC only ($p<0.0001$) (Table 3-6). The 3WAY provided a variety of plant residues that the RYE did not and the 3WAY also generally produced more biomass than the RYE. The differences in biomass characteristics of the two cover crops is likely the reason 3WAY had a more obvious effect on the soil health after three years as more diverse residue (Jian et al., 2020) and higher biomass are associated with soil health improvements (Smith et al., 2014; Wortman et al., 2012).

Conclusion

Biomass produced by both RYE and 3WAY cover crops doubled with each additional two weeks they were allowed to grow, but 3WAY outperformed the monoculture in biomass production at each time point. The amount of biomass a cover crop produces is correlated to many potential benefits such as creating a mulch to preserve soil moisture, building soil C and OM, improving soil aggregation and lowering BD. The results of this study support the link between cover crop biomass and soil health improvement as the highest biomass cover crop, the 3WAY, had the most impact on soil C, POXC, BD, and macro-aggregates. Another consideration for why the 3WAY had a stronger effect on soil health is that the 3WAY provided a variety of plant residues that the RYE did not. The higher accumulation of SOM has been linked to more diverse microbial communities driven by substrate–microbe interactions (Kallenbach et al., 2016). Soils under more complex crop rotations tend to accrue more C and microbial biomass (McDaniel et al., 2016), improve yields overall, and make agricultural systems more resilient under environmental challenges (Bowles et al., 2020). When cash crops are already rotated, multi-species cover crops provide a way to further diversify and more efficiently build SOM.

In the short three-year duration of cover crop treatments in this study, the effects were only statistically significant at the sandy site. In a review of no-till U.S. studies Blanco-Canqui (2021) found that sandy soils that have a lower starting SOC more readily accumulated SOC when cover cropped compared to finer textured soils that naturally include more C. Though the same trends of increasing SOC were observed at the silty site as the sandy, they were statistically indistinguishable after three years so it is possible that over a longer period, the treatment effects could become significant at the silty site as well. The lack of an obvious cover crop effect on soil health at the silty site highlights the need for more long-term studies when fine-textured soils are the subject.

Chapter 4 : Cover Crop Nitrogen Uptake is Unaffected by Fertilization Even in Depleted Soils

Abstract

Nitrogen (N) leaching occurs mainly from late fall through early spring when precipitation exceeds evapotranspiration. Uptake of N from deep soil before winter drives cover crop N capture, but deep rooting requires enough topsoil N for swift cover crop growth. Paradoxically, species effective for N-capture are most inhibited in N-poor soil. Our goal was to enhance cover cropping effectiveness in reducing N leaching, especially on sandy soils. Our objectives were to determine whether small fall N applications can increase N-capture by more than the amount of N applied and to develop soil nitrate criteria for evaluating where cover crop fertilization is justified. We conducted field experiments over 12 site-years using early-planted cover crops overseeded into corn (*Zea. mays*) on sandy soils. In 2020 we used large plots and farm equipment to spray cover crops in October with 0, 16, or 33 kg N/ha. In 2021 and 2022 we used paired (with and without N) 0.5 m^2 mini-plots and applied 0, 20, or 40 kg N/ha in September. We measured green ground cover at fertilization and again in December. In December we determined aboveground dry matter and N concentration. Fertilization did not consistently increase biomass or tissue N. Although there were slight increases in biomass, these were not correlated with soil nitrate concentration nor sufficient to increase N removal by more than was applied. Our results suggest that fall fertilization of cover crops will not improve cover crop N capture efficiency in sandy coastal-plain soils.

Introduction

The 2022 Ag Census (USDA/NASS, 2024) recorded a 39% increase in Mid-Atlantic USA hectares cover cropped between 2012 and 2022. While the practice is gaining popularity, most cover-cropped acres achieve minimal biomass, groundcover, and N-capture due to late planting and perhaps low residual surface soil fertility. Sedghi & Weil (2022) reported that having cover crop roots take up soluble N deep in the profile before the onset of winter is critical to capturing N and reducing nitrate leaching all winter. Planting cover crops in the Mid-Atlantic after early October is generally too late to clean up the deep soil profile before winter and is therefore ineffective in reducing N leaching. Only vigorously growing, early-planted cover crops can capture the deeper N before it leaches away over winter. Cover crops are capable of scavenging N from deep in the soil profile. Wang & Weil (2018) studying a corn silage (*Zea. Mays*) system on a Maryland dairy farm found that where an early–planted radish cover crop contained 120 kg N/acre in the above-ground biomass, the mineral N in the upper m of soil was depleted by only 40 kg N/ha, suggesting that the other 80 kg of N may have been taken up from a depth below the first meter. Hirsh & Weil (2019) subsequently studied soil under 45 mid-Atlantic crop fields and reported that residual end-of-summer mineral soil nitrogen in the upper 2 meters averaged 253 kg N/ ha. Some 55% of this residual N was found between 90 and 210 cm deep. In addition to early planting that allows enough growing degree days for deep rooting, effective capture of N deep in the soil profile also requires sufficient levels of available nutrients, especially N, for vigorous cover crop growth. Paradoxically, the most effective cover crop species for capturing excess N are also very responsive to N and do not grow vigorously in N-poor soils. Thus, cover crops may need N to capture N. Despite the large pool of plant available N in deep soil layers, topsoil may be depleted of plant-available N at fall cover crop planting time because of leaching, crop uptake, and immobilization. Immobilization and depletion of topsoil N are more of an issue after non-legume crops like corn that have a high demand for N. Low N in the topsoil in fall is especially likely on the sandy soils such as those common on the Mid-Atlantic Coastal Plain. Web Soil Survey (USDA/NRCS, 2019) indicates that such sandy soils cover approximately 170,000 ha of cropland in New Jersey, Delaware, and Maryland.

Assessing the Nitrogen Use Efficiency (NUE) is imperative for gauging the environmental efficacy of cover crop fertilization practices where reduction in N leaching is a priority. If N fertilization of cover crops is to enhance their ability to reduce N leaching, the target apparent NUE level should be > 100%, meaning that the boost from fertilizer should result in an increase in N uptake in the fall exceeding the amount of N applied so that the total N in the system susceptible to leaching is reduced, not increased. Apparent NUE is defined as:

$$\text{NUE (\%)} = 100 * (\text{kg N in fertilized plants} - \text{kg N in unfertilized plants}) / (\text{kg N applied})$$

Enhanced biomass production or a higher N concentration in plant tissues, or both concurrently, could contribute to increased nitrogen uptake and NUE. Since NUE for corn and other grains is typically less than 50% (Baligar et al., 2001), consistently achieving a NUE for cover crop fertilization > 100% may seem unlikely. However, when cash crops are fertilized in spring the large pool of deep soil soluble N has likely been already lost to leaching over the winter.

Apparent NUE > 100% has been reported. For example, Crusciol & Soratto (2009) in Brazil, documented an NUE of 128% when N was applied to a pearl millet cover crop preceding peanut cultivation, while other cover crop species exhibited NUE below 100%, predominantly attributable to insufficient increases in tissue nitrogen percentage compared to pearl millet. In Iowa, an investigation by Evans et al. (2019) compared fertilizing radish cover crops with surface-applied dairy manure versus incorporation of the manure before radish planting. Incorporating manure increased radish biomass threefold compared to unfertilized radish. The unincorporated manure treatments had an NUE between 3.31%-12.58% while the tilled in manure treatments had an NUE 5.32%-54.64%, assuming radish biomass contained 25 g N/ kg dry matter. Despite increases in biomass, they were not large enough to push NUE over 100%. Another study by Reiter et al. (2008) in Alabama applied N to a rye cover crop preceding cotton. Fertilizer applications two months before cotton planting resulted in NUE values of 135% and 97% in two of the study's three years, driven by both biomass and tissue N concentration responses. In a separate investigation in Alabama, Balkcom et al. (2018) also using cotton and rye cover crops, applied fertilizer or poultry manure to the cover crop in November or December increased rye biomass from 2,000 to 6,000 kg/ha. However, N tissue concentration remained low, resulting in a cover crop NUE of only 37%. Nitrogen fertilizer application in December or February in the Alabama studies may have been too late to provide enough growing degree days (GDD), slowing rye root assimilation of rapidly leaching N in deep soil layers. Despite the limited research on cover crop fertilization, considerable interest among farmers persists (Bechman, 2017; Dobberstein, 2016; Robison, 2012; Stewart, 2019).

We hypothesized that low N availability in the topsoil may stunt cover crops and prevent their roots from reaching the large pool of residual N deep in the soil profile before it leaches away to groundwater. We further hypothesized that small N fertilizer applications early in the fall would result in NUE exceeding 100% with the increased N uptake due to accessing N deep in the soil profile that more shallow-rooted unfertilized plants could not access.

Materials and Methods

Field Experiments

We conducted field experiments over 12 site-years using early-planted cover crops overseeded into corn (*Zea. mays*), mainly on sandy soils (Table 4-1and Table 4-2). In 2020, field experiments were conducted on two contrasting soils (loamy sand v. silty clay loam) at the Beltsville Facility of the University of Maryland Central Maryland Research and Education Center (CMREC) (39°00'46.0"N 76°49'35.7"W). Cover crops were interseeded into standing corn crop, to establish the cover crop early enough to reduce winter N leaching see (Sedghi and Weil, 2022; Sedghi et al., 2023). Main plots were 9.14 m wide and 54.9 m long and divided into 9.14 m wide x 18.3 m long subplots with three levels of N fertilizer spray-applied soon after corn harvest: 0, 17 and 34 kg/ha of N as Urea ammonium nitrate (UAN) solution. With the equipment available, fertilization was possible only after cash crop harvest, which left relatively few growing degree days for the 2020 cover crop to respond to the applied N. Therefore, in 2021 and 2022 we established field experiments with fertilizer hand-applied to paired 0.5 m^2 mini-plots in standing corn crops. These 2021 and 2022 experiments applied N earlier and included greater replication at CMREC and on commercial farms on the Eastern Shore of Maryland. All sites were in USDA plant hardiness zone 7b with mean annual precipitation of 112-117 cm. The soils at all sites had sandy loam to loamy sand textures in the A horizon (mainly Downer and Ingleside series), with the exception of a Christiana silt loam soil at one site in 2020. All fields had a history corn, soybean and small grain production, no-till management, annual winter cover cropping and minimal or no manure application. The weather patterns during the experiments at CMREC and on the Eastern Shore of Maryland are displayed in Figure 4-1 A-B (*PRISM*, 2004).

The CMREC experiments included plots of a three-species cover crop (radish *(Raphanus sativus* L.), crimson clover (*Trifolium incarnatum* L.), and cereal rye (*Secale cereale* L.)), as well as a cereal rye monoculture cover crop. Commercial farm sites on the Eastern Shore included a radish monoculture and a barley (*Hordeum vulgare* L.*)* - radish mix. Cover crops were sown before corn harvest at all site years. On the commercial farms cover crops were inter-seeded via airplane. In the 2020 experiment at CMREC the cover crops were sown using a Penn State-developed Inter-seeder (Youngerman et al., 2018) which drills three rows 19 cm apart between 76 cm apart corn rows at corn V5-6 stage in June. For the 2021 and 2022 experiments at CMREC a high clearance spray rig fit with an air-seeder and drop tubes was used to inter-seed the cover crops before corn harvest (Table 4-1).

Table 4-1 Characteristics and operation dates for all 16 study sites used in 2020, 2021, and 2022

Site	**Cover crop Species & Seeding Rate (kg/ha)**	**Seeding Method**
39D, 7E, 39A	112 kg/ha rye and a mix of 84 kg/ha rye, 4.5 kg/ha radish, 17 kg/ha crimson clover	In 2020 the PSU Inter-seeder Drill, afterwards Spra Coupe Sprayer (4640, Duluth GA) fitted Orbit Air Seeder (62 series 62DS12F, Owatonna, MN)
Lister	Barley 107 kg/ha radish 14 kg/ha	Airplane seeded
Holly, Zion, Duke, River	Radish 14 kg/ha	Airplane seeded

Microplot pairs were replicated 8 or 12 times. To delineate a pair of mini-plots, 0.5 m^2 rectangles made from PVC pipe were placed parallel to corn rows with the pairs 1 to 3 m apart in the same corn row middle. Micro plot pairs were delineated once cover crops had been planted and produced true leaves. A 0.5 L bottle of ammonium nitrate solution was sprinkled over the micro plot area to deliver 20 kg N/ha in 2021 or 40 kg N/ha in 2022. The no-nitrogen plots received 0.5 L of distilled water.

Table 4-2. Site characteristics and management over 12 site-years from 2020-2022

Site	Location	Reps	Cover Crop Planting Date	N Treatment Date	Biomass Sample Date	N Inputs During Previous Crop	Irrigation	Dominant Soil Series	Crops in 2020, 2021, 2022	Year
CM REC 39A	'-76.8328, 39.0144	4	6/26/20	10/21/20	12/13/20	0, 100, 160 kg/ha N at planting	Not Irrigated	Downer	Corn, Soy, Corn	2020
CM REC 7E	'-76.8328, 39.0144	4	6/27/20	10/23/20	12/13/20	0, 100, 160 kg/ha N at planting	Not Irrigated	Christiana	Corn, Soy, Corn	2020
CM REC 39A	'-76.8328, 39.0144	12	8/30/21	9/4/21	11/8/21	0, 100, 160 kg/ha N at planting	Not Irrigated	Downer	Soy, Corn, Soy	2021
CM REC 39D	'-76.8328, 39.0144	10	8/30/21	9/4/21	11/7/21	160 kg/ha N at planting	Not Irrigated	Downer-Hamonton Complex	Soy, Corn, Soy	2021
Holly Rd (H, HS)	-75.8468, 38.9407	4	8/20/21	9/11/21	11/20/21	162.5 kg/ha N at planting	Not Irrigated	Ingleside sandy loam	Soy, Corn, Soy	2021
Lister (L)	-75.8813, 38.9558	8	8/19/21	9/11/21	11/20/21	2722 kg chicken manure, 2722 kg compost, 90 kg/ha N at planting	Irrigated	Woodstown sandy loam	Soy, Corn, Soy	2021
Zion-Dry (ZD)	-75.8041,	4	8/20/21	9/11/21	11/20/21	162.5 kg/ha N at planting	Non-Irrigated	Hurlock sandy loam	Soy, Corn, Soy	2021
Zion-Irrigated (ZI)	-75.8041, 39.1211	4	8/20/21	9/11/21	11/20/21	162.5 kg/ha N at planting	Irrigated,	Hambrook sandy loam	Soy, Corn, Soy	2021

CM REC 39A	-76.8328, 39.0144	12 in each cover crop type	9/1/22	9/23/22	12/7/22	0, 112, 180 kg/ha N at planting	Not Irrigated	Downer	Corn, Soy, Corn	2022
CM REC 39D	'-76.8328, 39.0144	8	9/1/22	9/23/22	12/7/22	180 kg/ha N at planting	Not Irrigated	Downer-Hamonton Complex	Corn, Soy, Corn	2022
Duke	-75.843, 38.9677	8	8/15/22	9/17/22	12/2/22	162.5 kg/ha N at planting	Non-Irrigated	Hambrook sandy loam	Corn, Soy, Corn	2022
River	-75.8503, 38.9715	8	8/15/22	9/17/22	12/2/22	162.5 kg/ha N at planting	Non-Irrigated	Ingleside sandy loam	Corn, Soy, Corn	2022

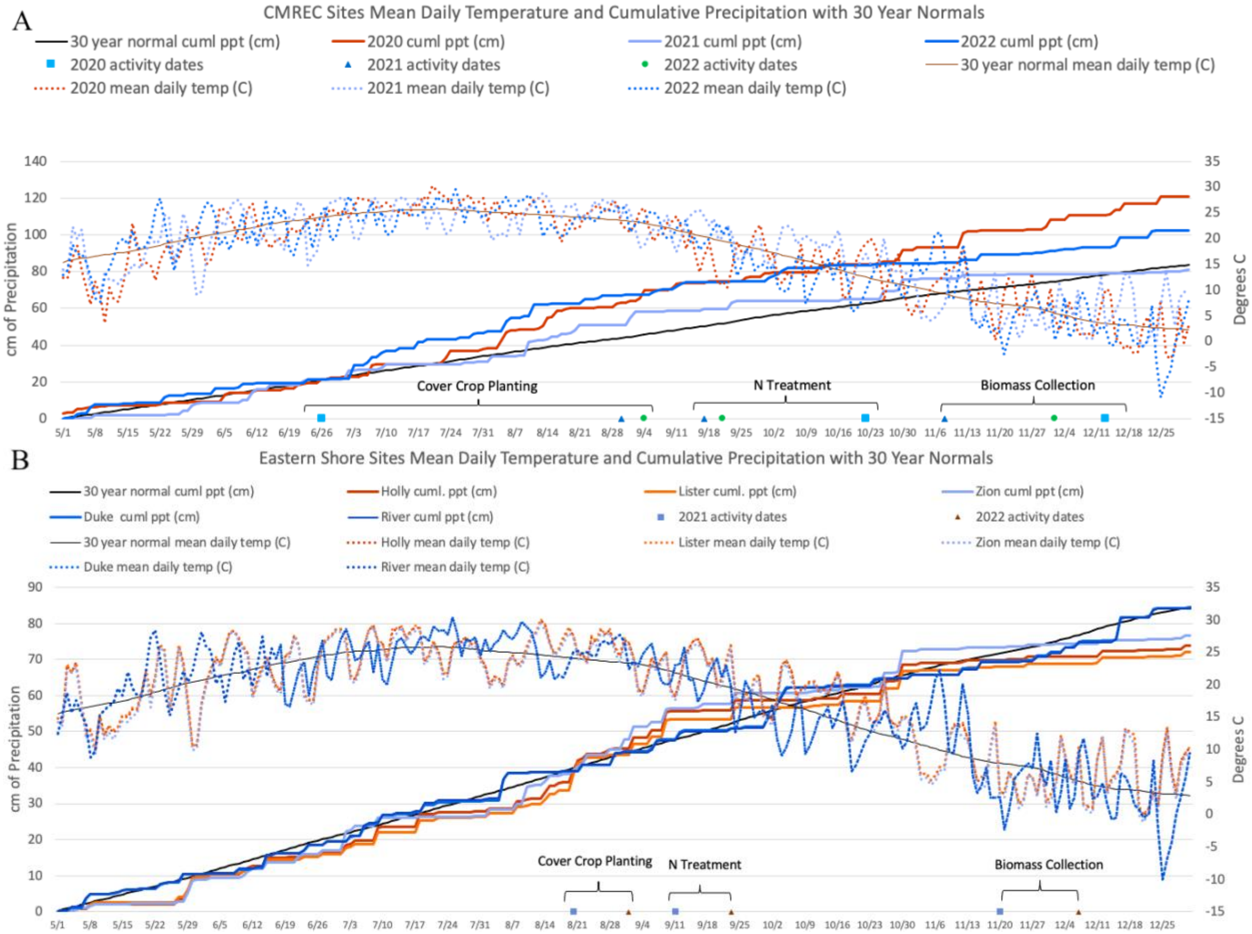


Figure 4-1. Panel A, cumulative precipitation and mean daily temperature at CMREC in Beltsville, MD from April to December in 2020, 2021, and 2022 compared to the 30-year normal. This location included sites 7E, 39A and 39D. Panel B, cumulative precipitation and mean daily temperature at each site on the eastern shore, MD from April to December in 2021 and 2022 compared to the 30-year normal.

Cover Crop Biomass and Soil Sampling

Green ground cover percentage was determined using the CANOPEO app (Patrignani & Ochsner, 2015) to find paired cover crop stands that did not differ more than 5% in green cover. Ground cover percentage was determined again in December using the CANOPEO app and the cover crops were harvested to measure biomass and N content by clipping them 1 cm above the ground and sorting them by species. Radish tubers were collected as well. The sampled tissue was dried at 18 ^{o}C to constant weight, the dry weight recorded, and the tissue ground in a Wiley mill (< 1 mm). The ground tissue was then analyzed for total N and C by dry combustion (LECO CN628 analyzer, St. Joseph, MI). At the time of fertilization, composite soil samples from 0-15 and 15-30 cm in depth were taken by collecting six cores in a circle 1 m from each plot pair to determine existing levels of mineral N. Soils were rapidly forced air-dried at room temperature and sieved to < 2 mm. We extracted the soil with 0.5 *M* K_2SO_4 (1:5 soil to solution ratio) and determined nitrate by the salicylic acid method (Cataldo et al., 1975) and ammonium by an indophenol blue analog method (Forester, 1995)). From the aboveground biomass dry weight and tissue N content we calculated the amount of N taken up per hectare as:

Dry matter (kg/ha) × N concentration (kg/kg) = N uptake (kg N/ha)

The response to N fertilization was calculated as:

N response = N uptake by fertilized CC – N uptake by unfertilized CC

Statistical analysis

We compared the N uptake difference between unfertilized and fertilized cover crops to the amount of N applied. We ran linear and non-linear regressions to determine any relationship between extractable soil NO_3-N or NH_4-N in 0-30, 0-15 or 15-30 cm depth and the cover crop N uptake, tissue N concentration and dry matter production response to fertilization. The significance of cover crop dry matter, green cover, change in green cover between September and December, tissue N content, and N uptake responses to fertilization was determined using a General Linear Models (GLM) analysis in SYSTAT 13 statistical software package (SYSTAT, 2022) with a split plot design for 2020 (cover crop type as the main plot and N application rate as the subplot factor). For the 2021 and 2022 mini-plot pairs, GLM was used with Sites, Blocks nested within Sites, and

Fertilization as categorical factors. Fertilization was considered a fixed effect, while sites and blocks were considered random effects.

Results and Discussion

In all 12 site-years of the study none of the cover crop growth parameters measured consistently responded to N fertilizer applied in early fall. Despite increases in fertilized cover crop biomass observed at some sites, the magnitude of the response was typically modest and not great enough to increase N uptake by an amount equivalent to the N applied.

Table 4-3. December cover crop responses to October N fertilizer application on both soils at CMREC in 2020. Effects on dry matter N g/kg and % green cover are shown with each cover crop type compared within the three N fertilizer levels with a p<0.05 denoted by a different lowercase letter. Effects on N tissue content and green cover % are compared with the two soil types combined. The effects on dry matter weight kg/ha are shown with each cover crop type compared within the three N fertilizer levels at each soil type with a p<0.05 denoted by a different lowercase letter.

Cover crop	**--------------- Fertilization rate ---------------**		
	0 N kg N/ha	**16 kg N/ha**	**34 kg N/ha**
Radish, three species mix dry matter g N/kg	21.0 a	23.0 a	16.0 a
Three species mix green cover %	14.7 b	17.1 ab	19.9 a
Rye green cover %	6.4 d	10.6 cd	11.9 c
Dry matter in three species mix on sandy loam soil kg/ha	1985 a	2000 a	2504 a
Dry matter in three species mix on silty clay loam soil kg/ha	1000 a	1110 a	1076 a
Dry matter of rye on sandy loam soil kg/ha	145.8 b	347.0 a	169.3 b
Dry matter of rye on silty clay loam soil kg/ha	245.6 b	469.3 a	689.5 a

The effect of post-corn-harvest N application on interseeded cover crop biomass measured in December 2020 (), shows trends towards higher biomass and % green cover with N application, but the N effect was significant only for the rye, especially on the silty soil. The response by the rye on the silty soil was similar in magnitude to the response trend for the mix on the sandy soil. The greatest significant increase in cover crop dry matter was from 245.6 to 689.5 kg/ha, an increase of 443 kg/ha of rye dry matter. The tissue N content of the radish cover crop was unchanged by fertilization (Table 4-3). Although we did not analyze the N content of the rye tissue, we can assume that N in the unfertilized rye tissue was approximately 15.0 g N/kg and even if the N in the fertilized tissue increase to as high as 25.0 g N/kg, the increase in N uptake would be only from 3.7 kg N/ha at 0 N applied to 17.2 kg N/ha where 33.6 kg N/ha was applied. This would represent an increase in N uptake of 13.5 kg N/ha (17.2-3.7), and an NUE of only 40%, far below the > 100% NUE that would be needed to justify fertilizing the cover crop to improve N capture. We speculated that the lack of a strong response to N in the 2020 treatments may have been due to the late timing of the N application after the interseeded cover crops had been growing for several months without any applied N and with few growing degree days remaining before winter dormancy. The study design was therefore changed in the following two years to allow earlier fertilizer application.

The N concentration in cover crop tissues was unaffected by fertilization throughout the three years of the study. Cover crop tissues had N contents ranging from 15 to 30 g N/ kg dry matter and differed mainly by site and species composition, rather than fertilization. Others have found cover crop plant tissue N concentrations changed as a result of N fertilization (Crusciol & Soratto, 2009). Though with rates much higher than those used in this study, N fertilizer increased tissue N content from 14.9 to 19.8 g N/kg. The lack of changes in tissue N concentration in our study could be attributed to the lower rates of fertilization used, but we also only examined the short-term response in the fall so 20 - 40 kg N/ha would have been enough to increase tissue N concentration in the small quantity of dry matter produced (generally < 1,000 kg/ha).

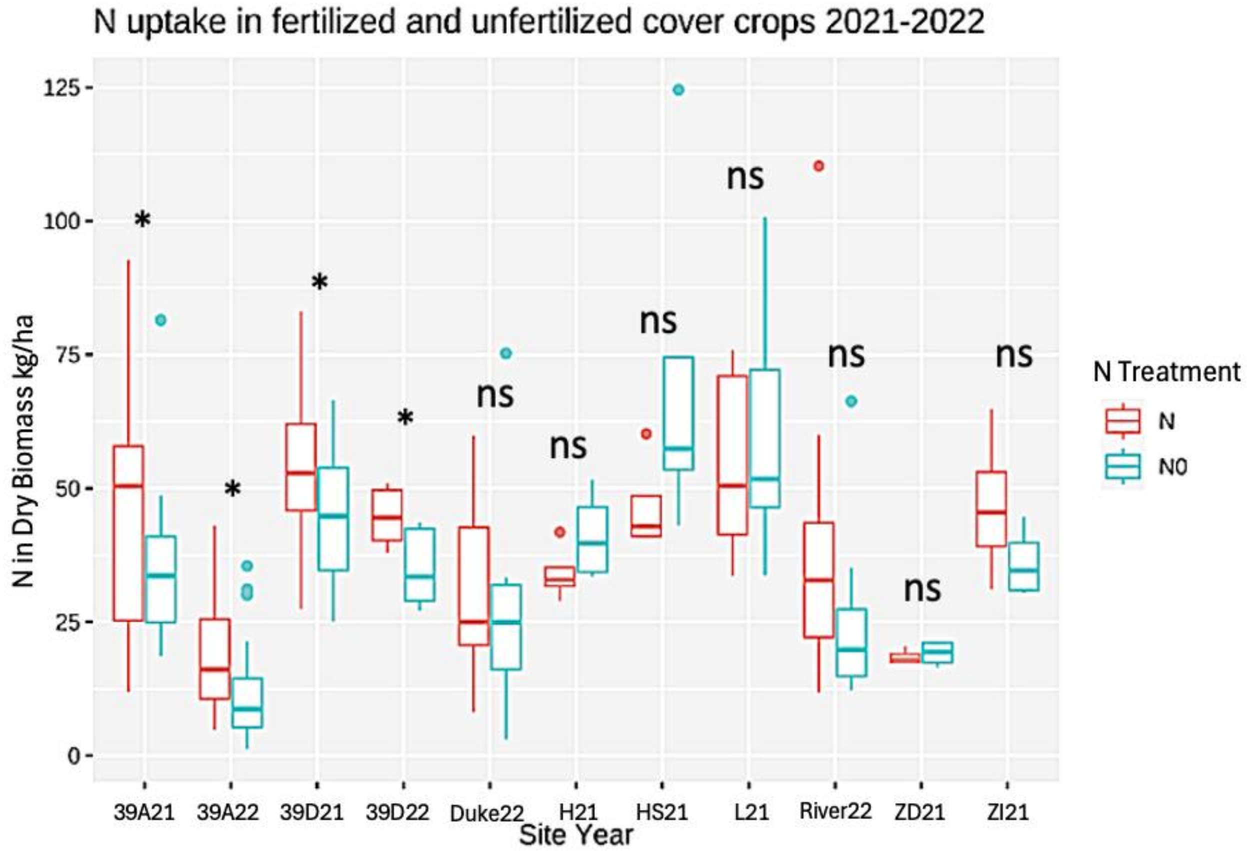


Figure 4-2. Nitrogen content in the total cover crop biomass averaged by nitrogen treatment at each site and year the site was included. In both years, the cover crops at sites 39A and 39D took up significantly more N when fertilized as compared to the unfertilized control (= p<0.05). At all other sites there was no statistically significant difference (ns) between the N content of fertilized and unfertilized control cover crop dry matter.*

In the absence of tissue N concentration changes, N uptake could be raised by increases in plant biomass. While biomass and consequently N uptake was typically higher in the fertilized cover crops, this was not always the case (Figure 4-2). In 2021 the overall effect of N fertilization on cover crop N uptake was not significant, with about as many sites showing an increase as those showing a decrease. Where the N uptake response for a site was significant, fertilized cover crops took up more N than unfertilized but in amounts that were smaller than expected. Even when the rate of N applied was doubled from 20 kg/ha in 2021 to 40 kg/ha in 2022, the difference between the N taken up by fertilized and unfertilized cover crops remained small. Average NUE of fertilized cover crops for sites with a significant fertilizer effect on N uptake were 50% in 2021 and 20% in 2022. A study that applied higher N applications to a radish cover crop in finer-textured soils in

Wisconsin found similar NUEs (Ruark et al., 2018). Where they found a significant effect from fertilization with dairy manure and with fertilizer, NUE was as low as 22% and did not get higher than 52%. Much like our results, the Wisconsin study found that the majority of site years showed that fall N applications had no effect on cover crop N uptake.

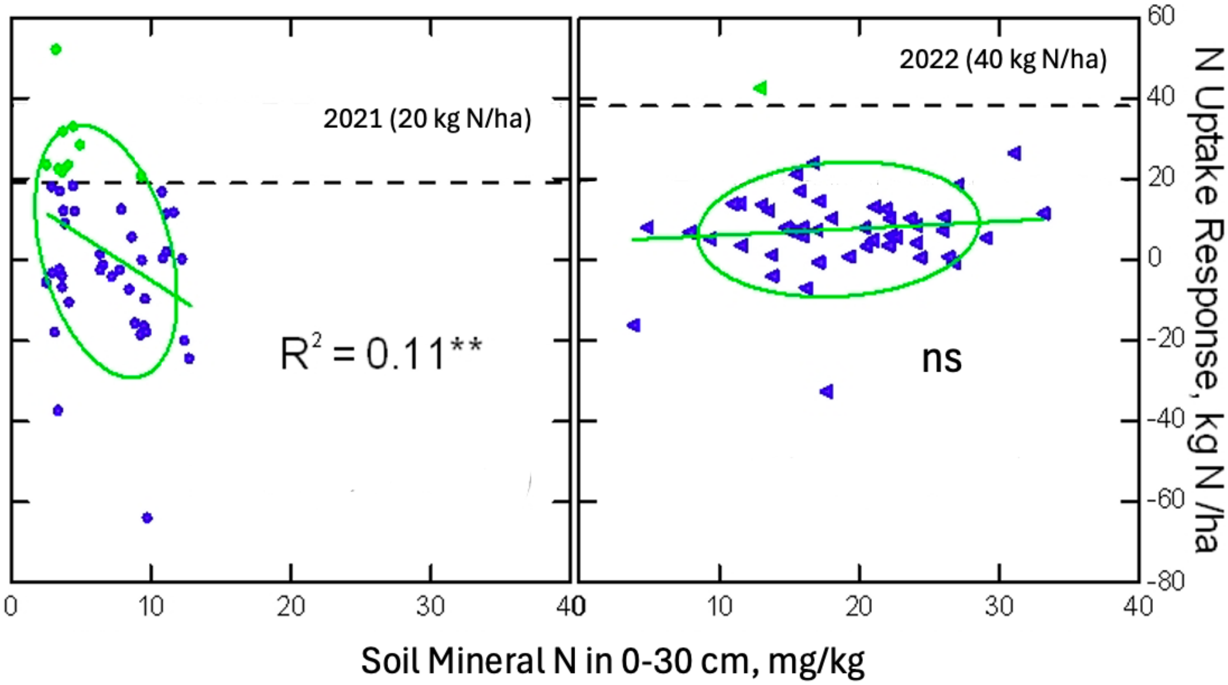


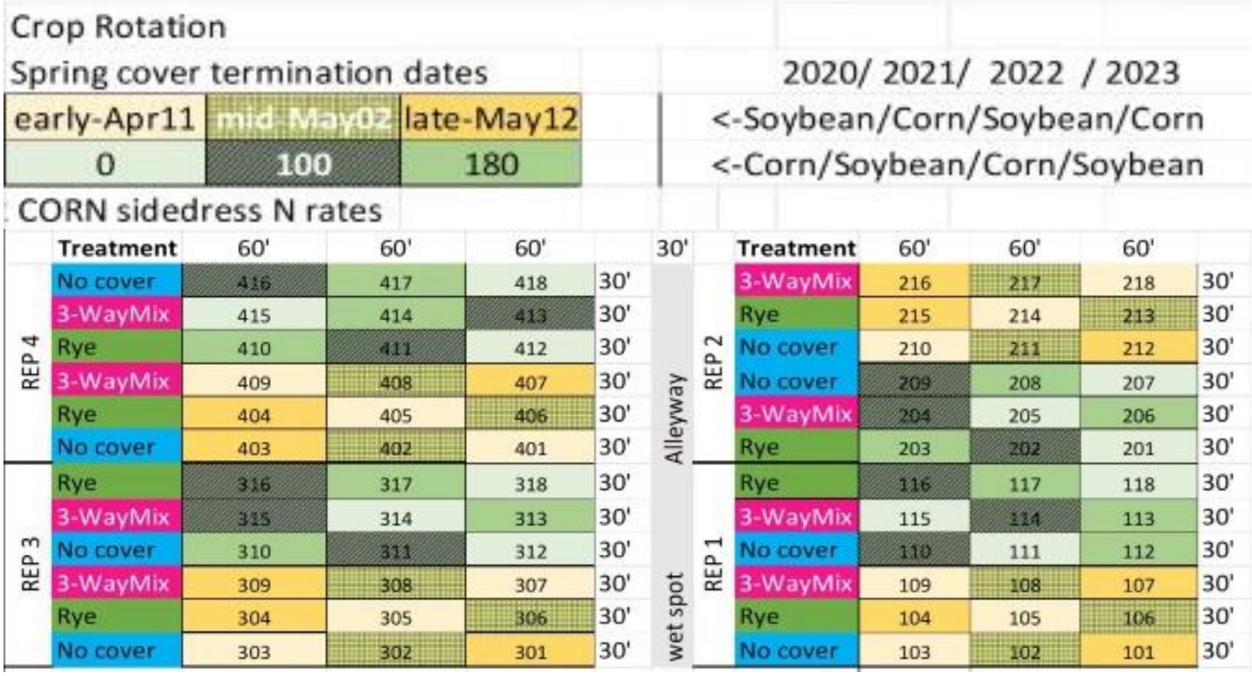
*Figure 4-3. The cover crop N uptake response for each plot pair (2021 on the left and 2022 on the right). Data points above the dashed line at 20 kg N/ha for 2021 and 40 kg N/ha for 2022 represent cover crop plots that took up more N than they received and are highlighted in green. In 2021 the N uptake response was weakly correlated with soil mineral N in the top 30 cm of soil with N uptake response decreasing as the soil N increases (R^2=0.11 **=$p<0.05$) but there is no such correlation in 2022 (ns).*

The N response of the cover crops in 2021 was higher than in 2022 and correlated, albeit weakly, with soil N, while there appeared to be no such correlation in 2022 (Figure 4-3). Since the initial soil N had no impact on the cover crops' growth response to added N in 2022, we speculate that cover crop growth in the fall was limited by factors other than N availability. The higher-than-average rainfall during the fall of 2022 when compared to the drier fall in 2021 may have created favorable conditions for denitrification and leaching and made detecting a response to N fertilizer more challenging. No significant rain events occurred in the week following fertilizer application in 2022 (Figure 4-1) and the absence of a correlation between cover crop biomass and soil N makes it unlikely that N availability was limiting growth. Overall cover crop growth was much greater in 2021 than 2022. In 2021, cover crop dry matter, regardless of treatment, was more than double that in 2022, likely due to cooler fall temperatures in 2022 compared to 2021. Average percent green cover was 44.5% in September of 2021 while it was 13.0% in 2022. A wider range of initial soil N levels (4-34 mg N/kg soil) were represented in the microplots in 2022 compared to 2021 (3-14 mg N/kg of soil). However, even in the most N-depleted topsoil (< 5 mg N/kg), most cover crops receiving fertilizer did not take up additional N equal to or exceeding that applied.

Conclusion

The reasons for the lack or small size of response to N application to the cover crops in fall are not clear. In the sandy soils that we studied; cover crops appeared to be more sensitive to environmental conditions other than to our N applications. We conclude that something other than low available N is limiting fall cover crop growth in sandy soils. Our results generally agree with the available literature on cover crop fertilization in that, while it is possible for cover crops fertilized in fall to achieve a NUE of over 100%, it is far more likely that NUE will fall short of 100%. Our data therefore do not support fall N fertilization of cover crops to increase N capture.

Supplemental Material



Crop Rotation

Spring cover termination dates

2020/ 2021/ 2022 / 2023

early-Apr11	mid-May02	late-May12	<-Soybean/Corn/Soybean/Corn
0	100	180	<-Corn/Soybean/Corn/Soybean

CORN sidedress N rates

	Treatment	60'	60'	60'		30'		Treatment	60'	60'	60'	
REP 4	No cover	416	417	418	30'	Alleyway	REP 2	3-WayMix	216	217	218	30'
	3-WayMix	415	414	413	30'			Rye	215	214	213	30'
	Rye	410	411	412	30'			No cover	210	211	212	30'
	3-WayMix	409	408	407	30'			No cover	209	208	207	30'
	Rye	404	405	406	30'			3-WayMix	204	205	206	30'
	No cover	403	402	401	30'			Rye	203	202	201	30'
REP 3	Rye	316	317	318	30'	wet spot	REP 1	Rye	116	117	118	30'
	3-WayMix	315	314	313	30'			3-WayMix	115	114	113	30'
	No cover	310	311	312	30'			No cover	110	111	112	30'
	3-WayMix	309	308	307	30'			3-WayMix	109	108	107	30'
	Rye	304	305	306	30'			Rye	104	105	106	30'
	No cover	303	302	301	30'			No cover	103	102	101	30'

Figure 2. The plot layout of the RCBD experimental fields at CMREC. The layout is the same at the Sandy and Silty fields. There are four blocks (REP 1-4), and each block contains soybean and corn main crops which switch places each year to make a corn-soybean rotation. Each main crop has plots with each cover crop type. Plots with soybean as the main crop that year had three different dates of cover crop termination and plots with corn as the main crop that year had three different N fertilization rates.

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