ABSTRACT

Title of Dissertation: DEEP SOIL NITROGEN CAPTURE AND

RECYCLING BY EARLY-PLANTED, DEEP-

ROOTED COVER CROPS

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Science and Technology

The overall purpose of this study was to improve the efficiency of nitrogen (N) cycling in Mid-Atlantic cropping systems through the use of cover crops. Our focus was on describing soil inorganic N pools (0-210 cm deep) and investigating the potential for cover crops to scavenge and recycle deep soil N. Few agronomic studies consider soil properties and processes deeper than the upper 20 to 30 cm, as the majority of roots, amendments, and practices such as fertilizer application or tillage occur on the soil surface or in the topsoil. We 1) assessed amounts of deep soil N on 29 farms in the Mid-Atlantic region, 2) used ¹⁵N tracer to investigate the capacity of various cover crops with early- or late-planting dates to capture and recycle deep soil N, and 3) investigated early-planted cover crop systems on 19 farm trials to assess their performance on farms with various soils with diverse management practices. We found that on average 253 kg N ha⁻¹ of inorganic N remained in the soil following summer crops, 55% from 90-210 cm deep. Soil following soybean had the same

amount or more of inorganic N than soil following corn throughout the soil profile. Using ¹⁵N isotopic tracer, we determined that radish, rye, and radish/rye mixes with and without crimson clover all could capture N from deep soil (60+ cm), but in order for cover crops to capture agronomically meaningful amounts of nitrate-nitrogen (NO₃-N) from deep soil, they had to be planted by early-September. Cover crop trials on 19 farms indicated that, while variable site-by-site, early-planted cover crops tended to accumulate substantial N in the fall and reduce residual soil NO₃-N levels substantially in the fall and spring. Cover crops also impacted subsequent corn growth and yield, with winter cereal tending to cause lower yields or increased corn N fertilizer needs compared to a no cover crop control, and forage radish sometimes leading to higher yields compared to the control. Overall, cover crops are effective at scavenging deep soil N in the fall, before winter leaching occurs, and under certain conditions, can release N for subsequent crops.

DEEP SOIL NITROGEN CAPTURE AND RECYCLING BY EARLY-PLANTED, DEEP-ROOTED COVER CROPS

by

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Chapter 1: Literature review

The following review will illustrate the importance of deep soil (1 m or more in depth) nitrogen (N) for improving nutrient use efficiency in agriculture and reducing N leaching loss to the environment. We will discuss the expected amounts of residual N in agricultural soil. We will next introduce cover crops, and discuss cover crop biomass and N accumulation, cover crop rooting dynamics, cover crop effects on residual soil N, and cover crop decomposition, nutrient release, and yield of subsequent cash crops.

Nitrogen dynamics in cropland

Nitrogen use efficiency in agriculture is a key issue for both maximizing farm profits and minimizing environmental impacts of farming. The N cycle is complicated, involving many inputs, outputs, and transformations. It is important to keep in mind that approximately 95-99% of N in the soil is in organic forms that are unavailable for plant uptake, and that plant available N, primarily the inorganic forms of nitrate (NO₃-N) and ammonium (NH₄-N), is released during microbial decomposition, which is dependent on various environmental and site-specific factors (Dahnke and Johnson, 1990; Weil and Brady, 2017). For example, the soil pH can enhance nitrification rates such that when soil pH increases 4.7 to 6.5, nitrification rates increase three to five times (Dancer, et al., 1973). Inorganic N forms can be leached, fixed on clay particles, and lost as gasses (N₂, N₂O, NO, NH₃). Van Meter, et al. (2016) found evidence from multiple long-term studies that total N (organic and inorganic) can accumulate over decades in fertilized row crop agriculture soils in the Midwest USA. In Iowa, assuming a linear rate of change, total N was estimated to have accumulated over 50 years at a rate of 31 kg ha⁻¹ yr⁻¹ in soils to 1

m depth. Total N increased in the 25-50 cm depth by 22%, in the 50-75 cm depth by 20%, and in the 75-100 cm depth by 14%. In Illinois, assuming a linear rate of change, total N was estimated to have accumulated over 45 years at a rate of 70 kg ha⁻¹ yr⁻¹ in the 0-100 cm deep soil. Total N increased in the 20-50 cm depth by 27% and in the 50-100 cm depth by 66%. Furthermore, an analysis of 2069 NCSS (National Cooperative Soil Survey) soil samples from the six sub-basins of the Mississippi River Basin found total N was estimated to have accumulated over 30 years at a rate of 55 kg ha⁻¹ yr⁻¹ in the 0-100 cm deep soil. Strickland, et al. (2015) found on loamy sand and sandy loam soils in Georgia, that incorporating conservation practices including a cereal rye (*Secale cereale* L.) and winter pea (*Lathyrus hirsutus* L.) cover crops, increased the amount of total N in soil (0-65 cm) on average 2000 kg ha⁻¹ over three years.

Regardless of fertilizer amounts, substantial residual soil nitrate-N (NO₃-N) and ammonium-N (NH₄-N) is found in the soil at the end of the crop growing season. On farms in central and southeastern Pennsylvania, in the fall following corn growth, when N was applied at economic optimum rates, there was on average 74 kg NO₃-N ha⁻¹ and 94 kg NO₃-N ha⁻¹ in the 0-120 cm deep soil for non-manured and manured sites, respectively (Roth and Fox, 1990). Under various wheat (*Triticum aestivum* L.) systems in a loam soil in Saskatchewan, Campbell, et al. (2006) found fall NO₃-N following wheat harvest in soil (0-2.4 m deep) was between 100 and 150 kg ha⁻¹, despite receiving fertilizer amounts based on soil tests. On a Willamette loam soil in Oregon, following an unfertilized winter wheat crop (in September), 0-120 cm soil had 44 kg NO₃-N ha⁻¹ and 32 kg NH₄-N ha⁻¹, and following an unfertilized broccoli (*Brassica oleracea* var. *italica*) crop, 0-120 cm soil had 34 kg NO₃-N ha⁻¹ and 26 kg NH₄-N ha⁻¹. With the recommended

rate of fertilizer, following winter wheat, 0-120 cm soil had 64 kg NO₃-N and 37 kg NH₄-N ha⁻¹, and following broccoli, 0-120 cm soil had 180 kg NO₃-N and 375 kg NH₄-N ha⁻¹ (Brandi-Dohrn, et al., 1997). In Minnesota, on a Webster clay loam soil, which had not been fertilized with inorganic N or manure for > 10 years, following an unfertilized corn crop, there was 71 to 91 kg N ha⁻¹ residual NO₃-N in the 0-3 m deep soil in the fall (Gast, et al., 1978). In the Northeastern USA, manured fields generally have higher residual N in 0-120+ cm soil and N loss due to leaching as opposed to non-manured fields (Angle, et al., 1993; Jokela, 1992; Roth and Fox, 1990; Weil, et al., 1990). In irrigated sandy soils in Maryland, Weil, et al. (1990) found that spring applications of manure to corn fields resulted in increased groundwater NO₃-N levels within one year of the manure application.

The proportion of NO₃-N to NH₄-N can vary widely. It is not uncommon, especially on manured soils, for NH₄-N concentrations to be as high or even higher than NO₃-N concentrations (Brandi-Dohrn, et al., 1997; Eghball, et al., 2004; Kristensen and Thorup-Kristensen, 2004b; Lacey and Armstrong, 2015; Sainju, et al., 2007). Greater NH₄-N levels could be attributed to ammonification exceeding nitrification due to higher soil water content or due to NH₄-N retention on clay particle cation exchange sites in the subsoil (Sainju, et al., 2007).

Previous studies have found NO₃-N levels to be more dynamic than NH₄-N levels. Kristensen and Thorup-Kristensen (2004b) found that October residual NO₃-N (0-2.5 m) varied between crop species, with sweet corn (*Zea mays* L. *Saccharata* Koern.) > carrot (*Daucus carota* L.) > white cabbage (*Brassica oleracea* L. convar. *Capitata*), whereas residual NH₄-N did not vary between the different species. From soil cores taken

in various barley (*Hordeum vulgare* L.), fescue (*Festuca* L.), and alfalfa (*Medicago sativa* L.) cropping systems (samples 1 m deep, six to nine times per year), Bergstrom (1986) found NH₄-N did not vary much between treatments, staying between 11 and 13 kg N ha⁻¹, whereas NO₃-N ranged between 23 and 68 kg N ha⁻¹. On a silt loam soil and a loamy sand soil in Wisconsin, Bundy, et al. (1993) found that spring soil NO₃-N (0-90 cm) was higher following soybean in a corn/soybean rotation than following corn in a nofertilizer continuous corn rotation, but there was no consistent effect of corn/soybean sequence on NH₄-N levels.

Soil inorganic N might be expected to increase following corn versus soybean cash crops since corn receives N fertilization, while soybean, a legume, does not usually receive N fertilization. However, previous studies found that corn did not have higher residual soil NO₃-N levels than soybean following crop harvest (Jaynes, et al., 2001; Pantoja, et al., 2016; Rembon and MacKenzie, 1997). In Nebraska on a Sharpsburg silty clay loam soil, Kessavalou and Walters (1999) found that May soil residual NO₃-N (0-150 cm) was lower in a continuous corn system than following corn in the corn/soybean rotation system, even though it was fertilized more often (every year) and had 25% less N removed in corn yield than the corn in the corn/soybean rotation. Mineral N may be higher following soybean than corn because the soil in a soybean crop is a high N environment with low C/N residues and high N root exudates. Microbial N immobilization, which would remove NO₃-N and NH₄-N from the solution, would be expected to be much lower under soybean than under corn. Green and Blackmer (1995) found that rates of N mineralization did not differ in soils having soybean residue from soils having corn residue. However, they suggested that there is higher N immobilization

following corn, due to the larger amount of corn residue than soybean residue, which allows N to be more available following soybean (Green and Blackmer, 1995).

Nitrate leaching and environmental concerns

The combination of cropping systems, the humid climate and weather patterns, and soil characteristics in the Mid-Atlantic USA make agricultural systems in this region prone to NO₃ leaching. A common cropping system in the Mid-Atlantic region is a corn (*Zea mays* L.) to soybean (*Glycine max* (L.) Merr.) rotation. In this rotation, from September to May there is no crop actively taking up N from the soil (Meisinger, et al., 1991), and little evapotranspiration (ET), but levels of precipitation remain equivalent to summer (Meisinger and Delgado, 2002). Leaching as NO₃ can be the main pathway for loss of N from farmland in the Mid-Atlantic, when there is little vegetation growing on cropland and precipitation is greater than ET (Meisinger, et al., 1991; Shipley, et al., 1992). The location of the residual N is of particular importance, as the deeper N is in the soil profile, the more likely it is to be lost through leaching or become inaccessible for following crop roots (Thorup-Kristensen, 1994). Nitrate that is leaching through the soil from August through May will likely be out of reach for the subsequent corn crop.

Nitrate leaching poses environmental risks, as NO₃ can enter groundwater and bodies of water, such as the Chesapeake Bay. According to the Chesapeake Bay Model, agriculture is responsible for approximately 43% of the N getting into the bay—17% from chemical fertilizer, 19% from manure, and 7% from air deposition of ammonia from livestock (e.g., emissions from poultry houses and dairies) and agricultural soil emissions (Environmental Protection Agency, 2010). Furthermore, in the Chesapeake Bay watershed, approximately 50% of the N load in streams was transported through

groundwater (Phillips and Lindsey, 2013). Excessive N and phosphorus (P) loading in the Chesapeake Bay and its tributaries have caused eutrophication—leading to harmful algal blooms, decreased water clarity, and decreased submerged aquatic vegetation—and periods of hypoxia (dissolved-oxygen concentration < 1.0 mg L⁻¹), stressing and killing aquatic organisms (e.g., shellfish; Ator and Denver, 2015; Phillips and Caughron, 2014).

Maryland law requires that farmers grossing > \$2500 year⁻¹ follow nutrient management plans, which indicate the nutrient sources (e.g., fertilizer, manure) that can be added to crops. The Maryland Department of Agriculture Nutrient Management Program intends to protect "water quality in the Chesapeake Bay and its tributaries by ensuring that farmers and urban land managers apply fertilizers, animal manure and other nutrient sources in an effective and environmentally sound manner" (Maryland Department of Agriculture, 2014). The Chesapeake Bay Watershed Implementation Plans (WIPs) indicate how the Bay jurisdiction states (Delaware, Maryland, New York, Pennsylvania, Virginia, West Virginia, and the District of Columbia) will meet Total Maximum Daily Load (TMDL) goals of reducing N, P and sediment inputs into the Bay watershed. The EPA (Environmental Protection Agency) Interim Evaluation of Maryland's 2016-2017 milestones reports that the Agriculture sector in Maryland was not on-track to reach its 2017 N target, which is a 60% reduction of the 2009 N loads into the Bay to achieve water quality standards. Specifically, 10 monitoring station sites indicated decreasing N load trends, two sites indicated no significant trend, and six sites indicated increasing N load trends (Environmental Protection Agency, 2017). Therefore, even with statewide, mandated efforts, N leaching continues to be a concern in Maryland.

It is important to note it is desirable to have plentiful N in the soil at certain times of year and in certain soil layers. Agricultural production is dependent on large pools of available N in the soil. However, how deep the N is located is key. Thorup-Kristensen (2006a) noted that the downward movement of N is not a loss process, but rather the loss occurs if the N leaches beyond the rooting zone of crops. In other words, N can move down through the soil profile, without actually being lost from the system, if it remains within the rooting zone of crops. Therefore, the loss of N from leaching is largely influenced by the amount of precipitation and infiltration (Thorup-Kristensen, et al., 2003).

Cover cropping to improve nitrogen use efficiency

Plants grown in the fall, following harvest of the cash crop, are called cover crops, catch crops, or green manures (Thorup-Kristensen, et al., 2003). Some cover crop species have the potential to quickly grow deep roots, and could serve as a "catch crop" to capture NO₃ in the fall months before it leaches out of reach, and potentially release N in the spring months to be used by the following cash crop (Dabney, et al., 2010; Meisinger, et al., 1990; Meisinger, et al., 1991). Cover crops can serve to reduce NO₃ concentration in aquifers used for drinking water and to decrease NO₃ concentrations in surface waters, lessening the risk of eutrophication and associated negative environmental effects (Thorup-Kristensen, et al., 2003). Cover crops can be fit within the framework of the existing crop system to scavenge and accumulate N in their tissue, and then through their decomposition, to supply N for subsequent crops.

Cover crops can improve the N use efficiency of a corn cropping system. Corn tends to scavenge the majority of its N from upper soil layers, especially after high

fertilization applications (Gass, et al., 1971; Ju, et al., 2007). On a loam soil, corn rooting depth was found to be < 0.8 m at V9 (nine leaf collar) stage and reached a maximum root depth of 1.2 m at silking stage (Zhou, et al., 2008). However, while corn roots did not reach depths > 1.2 m, subsequent winter wheat could use soil NO₃ up to 2 m deep (Zhou, et al., 2008). Using ¹⁵N tracer, Huang, et al. (1996) found that corn only removed 1 kg NO₃-N ha⁻¹ from 120 cm deep soil, whereas switchgrass (*Panicum virgatum* L.) removed 20 kg NO₃-N ha⁻¹.

Three main functional groups of cover crops that are grown include winter cereal grasses, legumes, and brassicas (Dabney, et al., 2010). In the current study, the three cover crop species studied in the ¹⁵N experiment, and in most of the on-farm trials, were forage radish (Raphanus sativus L.), cereal rye, and crimson clover (Trifolium incarnatum L.); however other winter cereal species were studied rather than cereal rye on some on-farm trials. Tribouillois, et al. (2015) found that the crop growth rate and N acquisition rate for forage radish was greater than crimson clover, which was greater than cereal rye. In Maryland, Dean and Weil (2009) found that early-planted forage radish contained 78-218 kg N ha⁻¹ (shoot plus root) and early-planted rye contained 43-112 kg N ha⁻¹ by late-fall. In Massachusetts on a Hadley fine sandy loam soil, forage radish planted in late-August to early-September had N accumulation of 128 kg ha⁻¹ (5730 kg ha⁻¹ dry matter) in the root plus shoot, and cereal rye with the same planting dates had N accumulation of 41 kg ha⁻¹ (2650 kg ha⁻¹ dry matter) (Jahanzad, et al., 2017). On a sandy loam in Denmark, forage radish N uptake (158 kg N ha⁻¹) was greater than cereal rye N uptake (91 kg N ha⁻¹) (Kristensen and Thorup-Kristensen, 2004a).

Cover crops are often N limited, as evident by their response to N fertilization. Cereal rye N content and biomass was positively correlated to fall N fertilizer application rate (Mirsky, et al., 2017; Komatsuzaki and Wagger, 2015) and residual soil N (Gabriel, et al., 2016; Hashemi, et al., 2013; Ruffo, et al., 2004). In the North Carolina Coastal Plain on a State fine sandy loam soil, the increase in cover crop biomass and N accumulation, with fall ammonium nitrate fertilizer applications compared to no fertilizer, was greater for earlier cover crop planting dates (Komatsuzaki and Wagger, 2015). Manure had a similar effect on cover crops as fertilizer. On a silt loam soil in Pennsylvania and loamy sand soils in Maryland, Ryan, et al. (2011) found that cereal rye biomass increased with poultry litter applications, but biomass did not increase with rye seeding rate. In southern Ontario, forage radish, perennial ryegrass (*Lolium perenne L.*), and oat (Avena sativus L.) cover crop biomass and N content was significantly higher in fall manured treatments (Thilakarathna, et al., 2015). Cover crops generally did not contain more N in their biomass than the amount applied as fertilizer (Mirsky, et al., 2017; Komatsuzaki and Wagger, 2015; Thilakarathna, et al., 2015). This provides evidence that while cover crops are often N limited, applying fertilizer to cover crops may reduce the overall N use efficiency of the cropping system.

Cover crops also respond to N fertilization on the previous corn crop. In Blacksburg, VA on a Hayter silt loam soil, Ditsch, et al. (1993) found that cereal rye biomass and recovery of residual fertilizer N increased with increasing fertilizer N applied to the prior corn crop. In Wisconsin, on a loamy sand soil, Bundy and Andraski (2005) found that cereal rye biomass was significantly higher when N fertilizer was applied to the previous corn crop compared to the corn with no fertilizer. Corn residue N

content and the C/N ratio was significantly related to rye biomass (Bundy and Andraski, 2005). Kessavalou and Walters (1997) found that in a corn/soybean rotation, the spring biomass and N uptake of the cereal rye following soybean was influenced by the N rate applied to the corn (approximately two years before). The response of rye biomass and N uptake to the previous year fertilizer rate was positive for one year and negative for one year of the study. (The negative response could not be explained by the authors). The amount of soil N availability at planting can also influence the domination of legume versus non-legume in a bi-culture mix (Möller, et al., 2008; Tribouillois, et al., 2016).

The amount and depth of N uptake during the fall months is determined by factors specific to cover crop species including the speed of cover crop establishment and growth, the rooting depth, and the cold tolerance (Thorup-Kristensen, et al., 2003). Nitrogen retention by cover crops was positively correlated with cover crop biomass (R²) = 0.53) and cover crop C/N ratio ($R^2 = 0.50$; Finney, et al., 2016). Planting cover crops earlier in the fall (allowing them to utilizer more growing degree days (GDD)) can significantly increase the capacity of cover crops to accumulate biomass and N across a range of soil types and geographic regions (Hashemi, et al., 2013; Ketterings, et al., 2015; Komainda, et al., 2016; Komainda, et al., 2018; Komatsuzaki and Wagger, 2015; Schroder, et al., 1996; Teixeira, et al., 2016). On a Hagerstown silt loam soil in Pennsylvania, Mirsky, et al. (2011) found that the spring biomass of rye or rye/hairy vetch (Vicia villosa Roth) mix cover crops planted on 25-August was 65% higher than the cover crops planted on 15-October. The loss in cover crop biomass from one date to the next increased through the fall dates (Mirsky, et al., 2011). Farsad, et al. (2011) found that small reductions in GDD had a large negative impact on rye biomass accumulation,

and estimated that delaying cover crop planting from the recommended planting date resulted in a 27% decrease in N accumulation for a one week delay, 29% decrease for a two week delay, 66% decrease for a three week delay, and 78% decrease for a four week delay. Vos and Van der Putten (1997) found a strong relationship between cereal rye and forage radish dry matter accumulation and intercepted radiation. Nitrogen uptake by brassica cover crops is more sensitive to growing season than is N uptake by monocots (Thorup-Kristensen, et al., 2003; Lacey and Armstrong, 2015). In a study at Rothamsted and at Woburn Experimental Farms in Bedfordshire, England that investigated winter wheat planting dates, Barraclough and Leigh (1984) found that September-planted wheat had over four times as much root dry weight and root length by March than Octoberplanted wheat. They found for the September planting, roots were present 1 m deep by December, but for the October planting, roots did not reach 1 m until April (Barraclough and Leigh, 1984). Earlier planting also reduces the depth of rooting required to "catch up" with NO₃ that is likely to be leaching deeper in the soil profile throughout the fall and winter. Farmers in Maryland typically do not plant cover crops until early- or mid-October (Maryland Department of Agriculture, 2018) after harvesting corn or soybean, often 1-2 months after the corn or soybean roots have stopped taking up NO₃ from the soil (Hanway, 1963; Ciampitti, et al., 2013).

Root depth and the rate of root growth are important factors determining whether plants are able to acquire N at the times when large amounts of it are available in the soil. Especially in environments with sandier soil or more precipitation, cover crops with fast growing roots may be the optimal system to capture NO₃ before it has a chance to leach to soil depths below the root zone. Measures of root depth, root frequency, and root

intensity (root intersections m⁻¹ line on minirhizotron) are all highly correlated with subsoil (0.5-1.0 m) NO₃ uptake (Thorup-Kristensen, 2001; Thorup-Kristensen, 2006a). Forage radish has been found to grow roots > 2.4 m deep (Kristensen and Thorup-Kristensen, 2004a) and have a depth penetration rate of 2 to 3.5 mm day⁻¹ °C⁻¹ (based on sum of daily average temperatures) (Kristensen and Thorup-Kristensen, 2004a; Thorup-Kristensen, 2001; Smit and Groenwold, 2005). Cereal rye has been found to grow roots 1.15 m deep (Kristensen and Thorup-Kristensen, 2004a) and have a depth penetration rate of 1.2 to 1.7 mm day⁻¹ °C⁻¹ (Kristensen and Thorup-Kristensen, 2004a; Thorup-Kristensen, 2001; Smit and Groenwold, 2005). Forage radish was found to have root frequencies (percentage of 4 x 4 cm crosses where roots observed on minirhizotron) > 40% down to 2.25 m deep (Thorup-Kristensen, 2006a). Forage radish reached 1 m deep with fewer GDD than cereal rye (Kristensen and Thorup-Kristensen, 2004a). While forage radish and winter wheat both reached a depth of approximately 2.5 m, forage radish reached this depth by early-winter but winter wheat did not reach this depth until late-spring (Thorup-Kristensen, et al., 2009). On sandy loam soils in Maryland, in 15-50 cm soil, forage radish had 1.5-2.7 times more roots than cereal rye under highly compacted soil, 1.1-1.9 times more roots than rye under medium compacted soil, and 0.8-1.2 times more roots than rye under non-compacted soil (Chen and Weil, 2010). Vos, et al. (1998) found that increased soil N supply decreased root length density (cm root cm⁻³ soil) of rye and forage radish cover crops.

Nitrogen uptake by cover crops is correlated with the reduction in NO₃-N leaching (Vos and Van Der Putten, 2004; Feyereisen, et al., 2006). A review of literature including studies with a range of soil types, climatic conditions, and tillage practices,

found that non-legume cover crops accumulated on average 20-60 kg inorganic N ha⁻¹ and reduced NO₃ leaching by on average 70%, in comparison to a no cover crop fallow treatment (Tonitto, et al., 2006). A meta-analysis using eight publications investigating cover crops on the Canterbury Plains of New Zealand (non-legume and legume cover crop species, all experiments on silt loam soils) found that cover crops took up on average 149 kg N ha⁻¹, reduced residual N following the cover crop by 34 kg N ha⁻¹ (57%), and reduced N leaching by 17 kg N ha⁻¹ or 50% (Teixeira, et al., 2016). A meta-analysis from Nordic countries investigating the effects of cover crops interseeding into spring wheat, barley, and oats found that non-legume cover crops reduced fall N leaching loss by 50% and soil inorganic or NO₃-N by 35%; legumes did not reduce N leaching (Valkama, et al., 2015).

In the Midwest, cover crops have been found to decrease fall and spring residual soil (0-60+ cm deep) NO₃-N (Lacey and Armstrong, 2015; Gieske, et al., 2016; Kessavalou and Walters, 1999) and NO₃-N leaching (Kaspar, et al., 2007; Strock, et al., 2004). Forage radish proved effective at reducing fall inorganic N, especially in deep soil layers (75-100 cm deep; Thorup-Kristensen, 1994) and reducing leaching (Justes, et al., 1999). In the spring, cereal rye also decreased residual fertilizer-derived N in each 30 cm soil depth increment from 0-90 cm compared to winter fallow (Ditsch, et al., 1993). In Maryland, Dean and Weil (2009) found that forage radish and cereal rye captured nearly all of the NO₃ in the soil to 1 m depth, while the no cover crop control plots, particularly in sandy soils, had large pools of NO₃ moving down between 60-90 cm. In the fall, the radish cover crop was more effective than cereal rye or rape (*Brassica napus* L. cv. Dwarf Essex) at depleting NO₃ from the soil and taking up N. In the spring, forage radish

had higher levels of soil NO₃-N from 0-60 cm than cereal rye (Dean and Weil, 2009). In Queen Anne's County, Maryland, Staver and Brinsfield (1998) found that a cereal rye cover crop following corn reduced annual leaching losses by 80% in comparison to no cover crop. In Beltsville Maryland, a study using tension-drained soil column lysimeters found that NO₃ leaching was reduced 95% in dry years and 50% in wet years for cover crops of cereal rye, wheat, or barley (Meisinger and Ricigliano, 2017).

Cover crop mixes can have the dual benefit of retaining N with a high-yielding non-legume cover crop, while also supplying N with a legume (Finney, et al., 2016; White, et al., 2017; White, et al., 2016). However, there is concern that including a N-fixing legume within a mixed species cover crop will impede the ability for the cover crop to scavenge soil NO₃. For example, prior to 2015, if farmers planted mixed-species cover crops that included a legume, they were not eligible for incentive payments through the Maryland Department of Agriculture cover crop program (Maryland Department of Agriculture, 2015). In order to overcome inherent trade-offs between the retention of N and supply of N by cover crops, cover crop and land management practices can be followed, such as planting cover crop mixtures with low non-legume seeding rates, maintaining low soil NO₃-N prior to cover crop planting, utilizing legumes that overwinter, and using non-legumes that are efficient at N retention (White, et al., 2017).

Often mixed species cover crops are as effective as monoculture cover crops in reducing residual soil N levels. For example, the amount of NO₃ in late-fall in the soil profile (0-90+ cm) was often the same for winter cereal or brassica cover crops with and without legumes, and always less than monoculture legumes (Couëdel, et al., 2018; Möller and Reents, 2009; Tribouillois, et al., 2016). A study using a mix of plant species

with different colored roots found that the maximum root depth and depth penetration rate of beet was not affected by the presence of legumes (Tosti and Thorup-Kristensen, 2010). Furthermore, the percent recovery in the fall and spring of surface applied fertilizer for a rye/clover mix was always higher than the clover monoculture and sometimes as high as rye monoculture (Ranells and Wagger, 1997).

Cover crops can encourage N mineralization. At a site in North Carolina with a State fine sandy loam soil, Komatsuzaki and Wagger (2015) found that under cereal rye, winter wheat, triticale (Triticum secale L.), and black oats (Avena strigosa L.), the change in soil inorganic N (0-90 cm) between fall and spring sampling dates were correlated with the accumulation of N in the cover crops, and the soil loss was always lower than the cover crop N uptake. They conclude that cover crops are effective N scavengers for both residual soil N, arising for example from previous crop fertilizer, and inorganic N formed from organic N mineralizing during the cover crop season. Alternatively, the change in soil inorganic N between fall and spring sampling dates for the no cover crop treatment (with winter annual weeds) was greater than the weed N accumulation. Cover crops have positive effects on soil microbial abundance and microbial processes (Blanco-Canqui, et al., 2015). Cover crops serve to add substrate for microorganisms throughout their growth through below-ground root exudation and turnover and above-ground leaf litter loss (Thorup-Kristensen, et al., 2003). Furthermore, agricultural practices, such as growing deep-rooted crops, can stimulate the decomposition of organic matter (Fontaine et al, 2007; Kuzyakov, 2010; Schmidt et al, 2011).

Cover crop decomposition, nutrient release, and yield of subsequent cash crops

The effect of cover crops on the subsequent crop yield varies with factors such as cover crop type, cover crop management (e.g., incorporation date), climatic variables, and the subsequent and previous crop types. Cover cropping cannot be equated to amending the soil, for example through adding manure or compost. Non-legume cover crops do not add N to the soil, but rather capture N from the soil and then return the N back to the soil (Thorup-Kristensen, et al., 2003). Nitrogen that is captured by cover crops can be a valuable resource for farmers, if it is released into the soil as available N in synchrony with cash crop N uptake needs (Dabney, et al., 2001). However, cover crops can have detrimental effects on the environment or agronomic system if cover crop N mineralization leads to increases in N leaching, or if cover crop N immobilization leads to increased fertilizer use on crops (Thorup-Kristensen, et al., 2003). There is sometimes a trade-off between N scavenging and N release. In Slovenia, Kramberger, et al. (2009) found that Italian ryegrass (Lolium multiflorum Lam.) and rape cover crops significantly depleted fall and spring soil inorganic N (0-90 cm), whereas subclover (Trifolium subterraneum L.) and crimson clover decreased soil inorganic N to a lesser extent and less frequently. However, the clovers tended to increase the following corn yield and corn N content, while rape had no effect on corn yield and corn N content, and Italian ryegrass had no effect or decreased corn yield and corn N content. To maximize cover crop N supply and provide the greatest yield benefit to a subsequent corn crop, cover crops should have low C/N ratio and high biomass N content (Finney, et al., 2016; White, et al., 2016). Species that fit these criteria, based on experiments performed in Pennsylvania, include legumes such as fava bean (Vicia faba L.), red clover (Trifolium pretense L.), and hairy vetch, grown in monoculture or in mixtures with each other or with grasses

including triticale, Italian ryegrass, or oat or a brassica forage radish. Thomsen, et al. (2016) found during incubation studies with forage radish, white mustard (*Sinapis alba* L.), and perennial ryegrass using a loamy sand soil that the residue C/N ratio and N concentration were the best single predictors for net N mineralization, regardless of temperature, or of cover crop type, age, or planting date.

A meta-analysis including 65 studies (grass included in 47 studies, legume included in 36 studies, mixture included in 13 studies) indicated that corn following a mixed cover crop had 13% higher average yields than corn following no cover crop, corn following a grass cover crop was not different than no cover crop, and corn following a legume cover crop had 21% higher yields than no cover crop (Marcillo and Miguez, 2017). Mixed cover crops with late termination dates (0-6 days before corn planting) had 30% higher corn yield compared to no cover crop (Marcillo and Miguez, 2017). While the corn yield response of cover crops in Canada, and the Great Plains and North Central regions of the USA were not significantly different from yield following no cover crop, the corn yield response in the Southeast and Northeast regions of the USA yielded 12-14% higher than no cover crop. Cover crops grown in northern regions will have shorter growth seasons and severe winters, which constrain their ability to accumulate biomass and N (Marcillo and Miguez, 2017).

Forage radish almost always winter-kills in Maryland and quickly decomposes, releasing inorganic N into the soil surface layers (0-60cm; Dean and Weil, 2009; Lounsbury and Weil, 2014). Jahanzad, et al. (2016) found that by week six of decomposition, as surface residue, forage radish had lost 60% of its initial N concentration while cereal rye had lost 30%. As buried residue, forage radish had lost

70% of its initial N concentration, while cereal rye had lost 40%. During the first 12 weeks of decomposition, soil at 20 cm, 40 cm, and 60 cm deep had higher NO₃-N concentrations in the radish treatment than rye treatment (Jahanzad, et al., 2017). On a Hadley fine sandy loam soil in Massachusetts, Jahanzad, et al. (2017) found that potato (*Solanum tuberosum* L.) yield and yield components were higher for potato following forage radish cover crop than cereal rye or no cover crop. Potato grown following forage radish produced the highest yield when fertilized with 75 or 150 kg N ha ⁻¹, while potato grown following no cover crop produced the highest yield when fertilized with 225 kg N ha ⁻¹ (Jahanzad, et al., 2017). In contrast, studies performed in Minnesota, Wisconsin, and Missouri indicated no fertilizer replacement value or benefit on corn yield of forage radish, despite substantial N uptake by the radish (Gieske, et al., 2016; Ruark, et al., 2018; Sandler, et al., 2015).

Nitrogen in winter cereal cover crops is released very slowly by decomposition and is often immobilized by microbes utilizing the abundant carbon in the residues and is therefore largely unavailable for crop uptake (Adeli, et al., 2011; Doran and Smith, 1991; Ketterings, et al., 2015; Thorup-Kristensen and Dresbøll, 2010). As a result, higher levels of spring N fertilizer are often applied following winter cereal cover crops than would be applied without a cover crop. Several studies have found negative yield responses to winter cereal cover crops. For example, in Iowa, on clay loam and loam soils, corn grain yield was reduced by 6% at the economic optimum N rate, and the negative effect of cereal rye cover crop on corn yield increased with rye cover crop biomass (Pantoja, et al., 2015). Adeli, et al. (2011) found that a cereal rye cover crop decreased cotton (Gossypium hirsutum L.) lint yield in comparison to no cover crop. However, negative

responses to rye are not consistent. In a corn/soybean rotation, corn grain yields following rye cover crop were 9.3% lower than yields following no cover crop in only one of the three years of the study, with no differences between yields in the other two years (Kessavalou and Walters, 1997). Kaspar and Bakker (2015) planted wheat, rye, or triticale cover crops before corn in a corn/soybean rotation in Iowa. Cover crops decreased corn yields in two of four years; however effects were different according to cover crop cultivars, and four rye cultivars did not significantly reduce corn yield. Some studies even found a benefit of winter cereal cover crops on following corn yield. In Pennsylvania, on a Hagerstown silt loam soil, Duiker and Curran (2005) found that a cereal rye cover crop did not impact or, if killed-early, may increase corn yields. In Massachusetts on a Hadley fine-sandy loam soil, Hashemi, et al. (2013) found that corn silage (that was not fertilized with N) yield was 34% higher following a cereal rye cover crop and 41% higher following an oat cover crop in comparison to a no cover crop control; however, these cover crops had 64 kg N ha⁻¹ applied to them at planting. Barley following a rye cover crop was found to have higher N supply than following no cover crop if the rye cover crop was incorporated early in the spring and there was heavy winter precipitation. Following dryer winters or later incorporated rye, barley had lower N supply than the no cover crop control (Thorup-Kristensen and Dresbøll, 2010). The overall consensus from meta-analyses is that negative responses from rye cover crops can be eliminated through management choices. Tonitto, et al. (2006) found that yields of cash crops that were fertilized at the recommended level were no different following nonlegume cover crops in comparison to a no cover crop control. A meta-analysis covering 47 studies concluded that grass cover crops had neutral effects on corn yield; however

management practices such as the corn N fertilizer rate was a highly significant moderator of yield response (Marcillo and Miguez, 2017).

Whereas N credits (extra N available for crop as a result of the cover crop) from grass cover crops are usually negative, requiring that additional fertilizer be applied to the following crop, legume cover crops result in fertilizer credits (reduced fertilizer rates) ranging from 56-135 kg N ha⁻¹ (Doran and Smith, 1991; Meisinger, et al., 1990). Legume cover crops foster microbial N fixation, which adds N to the system. Legume residues also have a relatively low C/N ratio (higher quality residue) which increases N availability following cover crop decomposition. Poffenbarger, et al. (2015a) found that at the end of the corn growing season, cereal rye had released only 8.5 kg N ha⁻¹, while hairy vetch had released 280 kg N ha⁻¹ and a 50/50 mix of rye/vetch had released 139 kg N ha⁻¹. For a hairy vetch/rye mix cover crop, as vetch went from comprising 0 to 100% of the mixture, the N content increased (64 to 181 kg N ha⁻¹) and C/N ratio decreased (83 to 16) (Poffenbarger, et al., 2015b). On sandy loam soils in North Carolina, Wagger (1989b) estimated that in one study after eight weeks of decomposition, rye released 24-26 kg N ha⁻¹, while crimson clover released 73-81 kg N ha⁻¹, and in another study after eight weeks of decomposition, rye released 8-33 kg N ha⁻¹, while crimson clover released 37-47 kg N ha⁻¹. The N uptake of corn following rye was 21-30 kg N ha⁻¹ less than corn following no cover crop, while the N uptake of corn following crimson clover was 41-45 kg N ha⁻¹ more than corn following no cover crop (Wagger, 1989a). The C/N ratio (Ranells and Wagger, 1997), N content in cover crop residue (Couëdel, et al., 2018; Seman-Varner, et al., 2017) and corn yield (Clark, et al., 1994) tended to be highest to lower in the order of legume > legume mixed with winter cereal or brassica > winter

cereal or brassica monoculture. While some brassica monocultures caused net N immobilization, the brassica/legume mixtures always resulted in net N mineralization. The cover crop mix of brassica/legume served to scavenge N, therein reducing the risk of N leaching, and to provide a green manure, therein reducing the risk of N immobilization and preemptive competition for the subsequent cash crop (Couëdel, et al., 2018). Studies have indicated that N fertilizer can be reduced for a potato crop following legume cover crops in comparison to winter wheat and cereal rye cover crops (Jahanzad, et al., 2017; Sincik, et al., 2008).

Preemptive competition can occur, if a cover crop takes up N that would have remained in the rooting zone of the subsequent crop in the absence of the cover crop (Thorup-Kristensen, et al., 2003). While residue mineralization will affect mostly the soil surface layers, which would affect the main crop early in the growing season, preemptive competition of N resources can reduce subsoil N, which could adversely affect the main crop later in the growing season (Thorup-Kristensen, 1993). The apparent effect of cover crops will depend on the soil depth considered. For example, examining 0-50 cm may result in very different conclusions than examining 0-150 cm. To minimize negative preemptive competition effects, the expected leaching intensity of the field and the rooting depth of the subsequent crop should be considered (Thorup-Kristensen and Nielsen, 1998).

The long-term goal of using cover crops is to sustain higher levels of production with less N loss, and therefore, the efficacy of cover crops may largely depend on choosing appropriate species according to the local hydrologic regime and minimizing preemptive competition (Thorup-Kristensen, et al., 2003). For example, Thorup-

Kristensen (2006a) observed that, in the spring, the subsoil (1-2.5 m) contained 120 kg N ha⁻¹ where no cover crop had been grown but only 49 and 60 kg ha⁻¹, respectively, where radish and Italian ryegrass cover crops had been grown. During the following crop season, they measured the available inorganic N in the root zone for each crop and the actual N uptake by each crop. They found that there was more available N and N uptake for leek (*Allium porrum* L.) after radish and leek after ryegrass in comparison to leek after no cover crop, and they found there was more available N and N uptake for beet (*Beta vulgaris* L. var. *esculenta* L.) after ryegrass (they did not investigate beet after radish) in comparison to beet after no cover crop. However, the N uptake for white cabbage was decreased following ryegrass or forage radish cover crop (Thorup-Kristensen, 2006a).

Practical considerations

Despite the fact that deeper N (1-2 meters deep) is most at-risk for leaching from the system, most studies only study the topsoil N. Cover crop studies often do not take soil samples deep enough to reveal differences in NO₃ depletion and cover crop root growth (Thorup-Kristensen, et al., 2003). Many studies investigating effects of cover crops on soil N (Ebelhar, et al., 1984; Kuo and Jellum, 2002; Ladoni, et al., 2015; Ruffo, et al., 2004; Sainju, et al., 2006) or the effects of other cropping practices on soil N (Anderson and Peterson, 1973; Poudel, et al., 2002; Rice, et al., 1986; Scalise, et al., 2015) have focused on the top 30 cm of soil.

There are challenges studying rooting patterns and nutrient uptake by plants deep in the soil. Shallow soil sampling may be due to the difficulty in obtaining deeper soil cores or the misconception that little N exists deeper in the profile and would also be

beyond the reach of roots. Deep soil coring is time consuming and laborious. In addition, soils and root systems are more heterogeneous in deeper layers than in topsoil layers. For example, measurements of soil organic carbon (SOC) had a higher coefficient of variation (80.2%) in the subsoil (30-40 cm depth) than in the topsoil (0-10 cm depth) (34.4%) (Usowicz and Lipiec, 2017). In addition, root intensity and root frequency is greatly reduced and therefore more spatially heterogeneous below 1 m deep (Kristensen and Thorup-Kristensen, 2004a). Therefore a greater number of core samples are needed to estimate parameters with confidence, but a smaller number of cores are usually dictated by logistical considerations. Root studies often underestimate root activity by not accounting for fine roots or root turnover with time (Dabney, et al., 2010). Methods of root density (cm cm⁻³) or intensity (cm cm⁻²) can differ depending on the methods used (e.g., core break, root wash, minirhizotron methods) (Wahlström, et al., 2015). For example, Wahlström, et al. (2015) found that measurements of forage radish root growth were higher for deeper soil layers using the minirhizotron method than the core break or root wash methods, which was attributed to preferential root growth and root branching along the minirhizotron tube. Nitrogen uptake by individual species within plant mixtures usually cannot be differentiated (Maeght, et al., 2013). Isotopic tracers can be used to assess uptake of applied nutrients from various depths (Hauck and Bremner, 1976; Maeght, et al., 2013). Injecting ¹⁵N, a nonradioactive heavy isotope, to a subsurface soil depth is a common method for assessing N uptake by crops or cover crops (Andersen, et al., 2014; Gathumbi, et al., 2003; Ju, et al., 2007; Kristensen and Thorup-Kristensen, 2004a; Kristensen and Thorup-Kristensen, 2004b; Ramirez-Garcia, et al., 2014; Yang, et al., 2014).

Cover crops often provide ecosystem services that are cumulative and not immediately measureable, and there is a need for long-term (> 5 year) cover crop studies to better understand the ecosystem services provided by cover crops and the year-to-year variability due to weather (Blanco-Canqui, et al., 2015). Over several years, the use of cover crops can lead to an increase in organic matter to the soil, which can lead to increased mineralization and an increase in plant-available N forms (Hansen, et al., 2000a). In Denmark, on a site with a coarse sand soil and long-term mean precipitation of 868 mm yr⁻¹, Hansen, et al. (2000a) investigated the long-term use of a spring-planted ryegrass cover crop undersown in spring barley or spring wheat. The long-term cover crop treatment had Italian ryegrass or perennial ryegrass grown for the 24 years. They found that long-term use of the cover crop could result in higher NO₃ leaching (on average 29% higher) than short-term cover crop use, especially when the cover crop was plowed into the soil in late-fall. Increased NO₃ leaching in long-term cover crop systems was accredited to increased mineralization and asynchrony between released cover crop N and crop needs (Hansen, et al., 2000a). Effects of long-term ryegrass use on increased NO₃ leaching were evident for at least four years following the discontinuation of cover crop use (Hansen, et al., 2000a). Furthermore, the long-term cover crop resulted in increased wheat yield, and allowed for wheat fertilizer to be reduced with no yield reductions, for over four years following the discontinuation of the cover crop (Hansen, et al., 2000a). A study examining the long-term impact of cover crops over 13+ years in Northern France found that cover crops increased total N stocks from 0-60 cm deep from 11.9-24.2 kg N ha⁻¹ yr⁻¹ (Constantin, et al., 2010). Cover crops also resulted in greater N mineralization and smaller N leaching losses that persisted during the 13-17 year period

(Constantin, et al., 2010; Constantin, et al., 2011). Chu, et al. (2017) found that only after three years, soybean yield was 15% higher after a multispecies cover crop mix (grasses, brassicas, clover) compared to a no cover control, while the corn or soybean yields of the first three years following the adoption of the cover crop were not different than the control. Hansen, et al. (2000b) found that introducing a perennial ryegrass cover crop following 25 years of residue removal in a field can cause spring wheat yield increases within two years, resulting in yields similar to the treatment with 24 years of cover crop use.

The availability of cheap fertilizer has obviated the need for legume cover crops to provide N nutrition for subsequent cash crops and probably is the main reason for the limited current utilization of cover crops in post-World War II agriculture (Thorup-Kristensen, et al., 2003). A survey of New York dairy farmers found that the primary reasons farmers discontinued the use of cover crops were time requirements and a delay in corn planting, and the primary reasons cited for farmers not adopting cover crop use included lack of time and the perceived high costs of planting cover crops (Long, et al., 2013). Challenges identified through focus groups for adoption of cover crops in Iowa included difficulty in timing of cover crop management (e.g., establishment in the fall and termination in the spring) within corn/soybean rotation systems and costs of establishing and terminating cover crops (Roesch-McNally, et al., 2017). Shorter season corn hybrids could allow farmers to plant cover crops earlier in the fall (Farsad, et al., 2011).

Conclusion

We hypothesize that considering deep soil N will improve our understanding of plant-soil nutrient cycling dynamics in agricultural systems. Deep soil N is the pool of N most at-risk for leaching and causing environmental problems. However agricultural research typically considers only the top 30-60 cm of soil as relevant to cropping systems. Through increasing our understanding of deep soil N cycling and the relevant environmental or management factors, we could improve the N use efficiency of agricultural systems. Cover crops have been shown to scavenge N to 2+ meters deep. Future work will evaluate various deep-rooted cover crop systems, which could capture and recycle the leftover inorganic N.

Chapter 2: Cropland soil profiles in the Mid-Atlantic contain large pools of residual inorganic N

Abstract

Summer annual crops are either fertilized with large amounts of N (e.g., corn) or they fix large amounts of N (e.g., soybean). In addition, organic matter is releasing N by mineralization during most of the year. We hypothesized that large amounts of mineral N remain in the soil following summer cash crops, particularly in deeper layers. We investigated the amount of mineral N remaining in the soil in September in the Mid-Atlantic USA for 14 fields with Coastal Plain sediment parent materials and 15 fields with Acidic or Calcareous rock parent materials by taking 210 cm deep soil cores. Across the 29 sites, total mineral N in the 0-210 cm profiles ranged from 87.4 to 515 kg N ha⁻¹, with an average of 253 kg ha⁻¹. Of the 253 kg ha⁻¹, 45% was NO₃-N and 55% was NH₄-N. The soil layers from 0-30 cm, 30-90 cm, 90-150 cm, and 150-210 cm, contained 22%, 23%, 27%, and 28%, respectively, of the profile mineral N. We took deep soil cores in side-by-side corn-soybean fields in September, and found significantly higher levels of NO₃-N following soybean than following corn, but similar levels of NH₄-N. The deeper the mineral N is in the profile, the greater the risk that it will leach out of the soil and into groundwater over the winter. The pool of residual deep soil N could serve as a valuable resource for farmers if cover crops could capture and bring it to the surface where it could be recycled to subsequent crops and potentially allow farmers to decrease fertilizer N inputs.

Introduction

Nitrogen (N) use efficiency in agriculture is a key issue for both maximizing profitability and minimizing environmental impacts of farming. In the Mid-Atlantic region of the USA, N leaching is prevalent, due to the combination of the crops grown, climate, and soils. Corn (*Zea mays* L.) (for grain or silage) and soybean (*Glycine max* (L.) Merr.) are the highest land area annual crops in Mid-Atlantic Region (USDA Census of Agriculture, 2012). Corn typically stops taking up N from the soil by early-September (or 100 days after emergence) when corn maturity is approached (Hanway, 1963; Ciampitti, et al., 2013). Furthermore, the region has a humid temperate climate, in which most leaching losses occur during the non-growing season (winter and early-spring), while there is little evapotranspiration (ET) but levels of precipitation equivalent to summer (Meisinger and Delgado, 2002).

Excessive N and phosphorus (P) loading in the Chesapeake Bay and its tributaries have caused eutrophication, leading to harmful algal blooms, decreased water clarity, and decreased submerged aquatic vegetation, and periods of hypoxia (dissolved-oxygen concentration < 1.0 mg L⁻¹), stressing and killing aquatic organisms (e.g., shellfish; Phillips and Caughron, 2014; Ator and Denver, 2015). Largely due to environmental concerns related to the Chesapeake Bay, in 1998 the Maryland legislature established the Maryland Water Quality Improvement Act (WQIA), requiring growers to implement nutrient management plans based on N and P (Parker, 2000). Farming operations grossing more than \$2500 year⁻¹ must follow approved nutrient management plans, which indicate the nutrient sources (e.g., fertilizer, manure) and amounts that can be added to crops (Maryland Department of Agriculture, 2014). In addition, Chesapeake Bay

Watershed Implementation Plans (WIPs) have been developed to indicate how the states in the Chesapeake watershed (Delaware, Maryland, New York, Pennsylvania, Virginia, West Virginia, and the District of Columbia) will meet Total Maximum Daily Load (TMDL) goals of reducing N, P and sediment inputs into the Bay (Environmental Protection Agency, Chesapeake Bay TMDL). A recent evaluation (Environmental Protection Agency, 2017) concluded that the Agriculture sector in Maryland was not ontrack to reach its 2017 water quality target of a 60% reduction from the 2009 N loads into the Bay. Therefore, even with statewide, mandated efforts, N leaching continues to be a concern in Maryland.

The spatial and temporal patterns of N in the soil profile influence whether N is accessible to crops during the crop growth period or likely to be lost. Nitrogen remaining at the end of the growing season, especially in deeper soil layers is of particular concern. The deeper the N is in the soil profile, the more likely it is for winter leaching to move it below the root zone of following crops or out of the soil profile and into the groundwater (Thorup-Kristensen, 1994). Many measurements of soil N are only for 30 cm or less depth (Anderson and Peterson, 1973; Chu, et al., 2017; Ebelhar, et al., 1984; Kuo and Jellum, 2002; Ladoni, et al., 2015; Poudel, et al., 2002; Rice, et al., 1986; Sainju, et al., 2006; Scalise, et al., 2015). For example, Poudel, et al. (2002) investigated effects of various farming systems (e.g., with organic vs conventional practices) on soil mineral N, but only looked to 30 cm deep, and Chu, et al. (2017) reported that a multispecies cover crop mix increased soil inorganic N, but only took soil cores to 15 cm deep. Limited information is available on mineral N in deeper soil layers, especially deeper than 1 m. Studies that have investigated deep soil N indicate that even when crops were fertilized at

recommended rates, substantial mineral N apparently remained in the soil profile at the end of the crop season. For example, on farms in central and southeastern Pennsylvania, in the fall following corn growth, when N was applied at economic optimum rates, there was on average 74 and 94 kg NO₃-N ha⁻¹ in the 0-120 cm soil profile for non-manured and manured sites, respectively (Roth and Fox, 1990). Furthermore, we hypothesized that substantial residual N remains in the soil profile following cash crops due to the performance of early-planted cover crops. Fall cover crops have been documented to capture high amounts of N. For example, in Maryland, Dean and Weil (2009) found that early-planted brassica and rye cover crops captured 36-100 kg N ha⁻¹ and 99-171 kg N ha⁻¹ following corn and soybean, respectively. We therefore saw a need to conduct a survey of farm fields to assess the size of the pool of residual mineral N remaining in Mid-Atlantic cropland soils after summer crop uptake had ceased.

Cover crop systems are widely used, especially in Maryland and Delaware due to state-funded cash incentive programs (Maryland Department of Agriculture, 2018). Some cover crops have the potential to scavenge residual N from the soil profile, and could serve as a "catch crop" to capture NO₃ in the fall months before it leaches out of reach (Dabney, et al., 2010; Meisinger, et al., 1990; Meisinger, et al., 1991). However, a study from Beltsville, MD found that to scavenge N during fall months, cover crops must be planted by mid-September (chapter three). Currently, cover crops in the region are typically not planted until October or November, and have most of their growth in spring months. We reasoned that these late-planted cover crops would not prevent NO₃-N leaching if significant amounts of mineral N remain following cash crops in deeper layers. Investigating the amount and location of residual fall N can allow farmers and

policy-makers to make informed decisions about optimal cover cropping practices. For example, if a large pool of mineral N were present in the fall, farmers may be motivated to use early-planted cover crops to capture and gain economic value from some of that N by bringing it to the soil surface.

The current study investigated pools of mineral—nitrate-N (NO₃-N) and ammonium-N (NH₄-N). However, it is important to note that approximately 95-99% of N in soils is in organic forms that are unavailable for plant uptake, and that NO₃-N and NH₄-N are released from these organic forms during microbial decomposition, which is dependent on various environmental and site specific factors (Dahnke and Johnson, 1990; Weil and Brady, 2017). In addition to being taken up by plants, mineral N forms can be leached, held on clay particles, and lost as gasses (N₂, N₂O, NO, NH₃).

In the current study, our specific objectives were:

- Investigate the amount and depth of mineral-N in soil profiles after summer crop
 N uptake had ceased in the Mid-Atlantic region.
- 2) Determine differences between the residual NO₃-N and NH₄-N in total amounts and depth distribution in the upper 210 cm of soil.
- 3) Compare pools and depth distribution of residual NO₃-N and NH₄-N among soils formed from Coastal Plain, Acidic rock, and Calcareous rock parent materials.
- 4) Compare pools of mineral-N following corn versus following soybean crops.

Materials and Methods

Location

We sampled soil to 210 cm deep on a total of 29 farm fields, on a wide range of commercial farm row-crop fields, in August-September during a three year period (2014-2016). The timing of the samples was chosen to determine the amount of N left in the profile after summer cash crop N uptake had ceased.

Soil was sampled in this survey across the main agricultural regions in Maryland and southeast Pennsylvania, in the Piedmont and Ridge and Valley physiographic regions of Maryland and Pennsylvania and the Coastal Plain physiographic region of Maryland (Figure 1; Table 1). The Coastal Plain region, which extends inland from the Atlantic Ocean and estuaries, tends to be flat and composed primarily of sedimentary rock (Polsky, et al., 2000), having multiple levels of unconsolidated to weakly consolidated acid sands and clays (Ciolkosz, et al., 1989). The Piedmont falls within the foothills of the Appalachian mountain range, to the west of the Coastal Plain. This region is composed primarily of metamorphic and igneous rock (Polsky, et al., 2000). The bedrock is primarily granite and schists, with lowland insets of red shales and sandstones (Ciolkosz, et al., 1989). The Ridge and Valley region falls to the west of the Piedmont, and consists of a folded terrain with several parallel, eroded mountains, and contains mostly sedimentary rock (Polsky, et al., 2000). The bedrock in the ridges is primarily sandstone and in valleys is primarily shale and limestone (Ciolkosz, et al., 1989). The Piedmont terrain is erosional with soils typically less than 1 m deep to rock, having higher clay content and lower sand content than Coastal Plain soils. Piedmont soil infiltration rates are typically 6-15 cm h⁻¹. Coastal Plain soils tend to be deeper with soil infiltration rates of 13-28 cm h⁻¹ (Markewich, et al., 1990). The sandier textures and higher infiltration rates common of many soils in the Coastal Plain region surrounding

the Chesapeake Bay allow NO₃ to leach more rapidly in comparison to the finer textured soils of the Piedmont areas.

The 29 sites were classified into three groups (Table 1), based on soil parent materials: 1) soils formed from coastal plain sediments (Coastal Plain), 2) soils formed from acidic rock parent materials (Acidic), and 3) soils formed from calcareous rock parent materials (Calcareous). In order to provide a representative sample of typical agriculture in the region, most farms practiced no-tillage or limited-tillage, there was a range of manure histories, and sampling on most farms followed corn or soybean crops, although other crops were included, which were common to particular counties (e.g., tobacco). Eight of the 29 fields were selected as four pairs of side-by-side corn and soybean fields, in order to evaluate the effect of previous crop on residual N. The fields that were paired were physically located next to each other and also were in the same soil series. Site descriptions for soil core transect sites, indicating crop, manure, and tillage history, and mapped soil series are given in Table 1. Appendix 1, Table 5 lists soil pH, percent sand, percent clay, percent C, and percent N of the study site soils for each 30 cm depth increment from 0-210 cm, and percent soil organic matter (SOM), and P, K, Mg, Ca, and S (mg kg $^{-1}$) for 0-30 cm.

The study region has a humid climate with annual rainfall relatively uniformly distributed throughout the entire year (Maryland Department of State Planning, 1973). From 1895-1997, the Mid-Atlantic Region had an average annual temperature of 11°C and average monthly precipitation of 87 mm (Polsky, et al., 2000). The climate in the study region varies according to the physiographic configuration, with Coastal Plain

average temperatures 1-2° C warmer than Piedmont and Ridge and Valley Maryland temperatures (Planning, 1973).

Arrangement of soil cores in surveyed fields

In 2014, soil cores were collected along a straight transect going down the slope of the field. Two soil cores were taken at five points along the transect (Figure 2). The two soil cores were spaced 60 cm apart.

In 2015, soil cores were taken at four points in the field, one in each of the four blocks of the anticipated future cover crop experiment. At each point, three cores were taken, in three positions relative to the crop stubble—in the row ("row"), 19 cm from the row ("side"), and in the center between two rows ("center") (Figure 2). Soil was sampled in this way in order to investigate if the position of the soil core, relative to where the N fertilizer may have been applied during June side-dressing, affected the soil N concentrations, in order to address concerns that soil N was being overestimated due to the soil core placement.

In order to test for differences between row, side, and center soil core positions, an analysis of variance (ANOVA) was performed with soil core position, soil depth, and the interaction of core position x depth as independent variables and the soil NO₃-N or NH₄-N as a dependent variable across the seven farms (with rep within farm as the random variable). For soil NO₃-N, there was no significant effect for position (p = 0.5594) or position by depth (p = 0.9639). For soil NH₄-N, there was no significant effect for position (p = 0.5593) or for position by depth (p = 0.9639). This provided evidence that soil NO₃-N or NH₄-N was not being overestimated due to sample core placement.

In October 2016, soil cores were taken in four sets of side-by-side corn and soybean fields (site identification numbers 14, 15, 16, 17, 26, 27, 28, 29 on Figure 1). The side-by-side corn and soybean fields were sampled on the same day. At some locations, soil samples were taken after corn was harvested but soybean was still in the field (dry and mature). Soil cores were collected along a straight transect going down the slope of the field; two soil cores were taken at five points along the transect. The two soil cores were spaced 75 cm apart. Soil cores were both taken in the crop row between two corn plants or between two soybean plants.

Soil sampling and analysis

Soil cores were taken by hand driving Veihmeyer probes into the ground using a 6.8 kg drop hammer (Veihmeyer, 1929; Dean and Weil, 2009). Cores were taken from 0 to 210 cm deep when possible, or until the probe hit an impassible layer of rock or hit groundwater. The available equipment and resources did not allow soil cores to be taken deeper than 210 cm. In 2014 and 2016, soil was divided into 15 cm increments and two soil cores taken from each point along the transect were composited for each depth increment. In 2015, soil was divided into 30 cm increments and no cores were composited. Detailed procedures of soil sampling from each year can be found in **Appendix 2**. The collected soil was put into sealed plastic bags and stored in a cooler with ice for transport to the lab. The soil samples were dried at 40 °C for at least 48 hours, and the soil was sieved through a 2 mm sieve. The weight of the soil at the field moisture level, the weight of the soil after drying, and the weight of the gravel that did not pass through the 2 mm sieve was determined.

Exchangeable NO₃ and NH₄ in the soil was extracted with 0.5 *M* potassium sulfate (K₂SO₄) solution. Two grams of dry soil were mixed with 20.0 ml of 0.5 *M* K₂SO₄ in 50 ml tubes. The tubes were shaken horizontally at 200 rpm for 30 minutes and then allowed to settle in a vertical position for 10 minutes. The supernatant liquid from the tubes was filtered through VWR 410 filter paper. The filtrate was tested for NO₃-N and NH₄-N using a Lachat QuikChem 8500 Automated Ion Analyzer (Hach Company, Loveland, CO). The filtrate was analyzed for NH₄-N by the salicylate method and for NO₂-N and NO₃-N by cadmium reduction method. The measured NO₃-N and NH₄-N (mg NO₃-N L⁻¹ or mg NH₄-N L⁻¹) was blank-corrected with filtered 0.5 *M* K₂SO₄ solution samples and converted to mg NO₃-N or NH₄-N kg soil⁻¹ (**Appendix 3**).

In order to convert values of NO₃-N and NH₄-N concentrations in the soil to stock amounts of NO₃-N and NH₄-N in kg ha⁻¹, soil bulk density values were estimated from dry mass of known soil volumes in the cores and corrected for gravel content (**Equation 1**). The mass and volume of soil was determined for each of the soil cores taken with the Veihmeyer probe. Bulk density values for each farm were based on the average of all cores from that farm for a given depth increment (e.g., 0-120 cm or 120-210 cm) (**Appendix 4**).

Equation 1 Bulk density of soil

$$\frac{g \, soil}{cm^3} = \frac{(g \, soil + gravel) - g \, gravel}{(\pi r^2 * height) - (\frac{g \, gravel}{2.65 \, g \, cm^{-3}})}$$

Where,

r = radius (in cm) of soil core, as determined by measuring the inside diameter of soil core tip to three significant figures and dividing by 2.

height = length (in cm) of the increment of soil collected estimated bulk density of gravel = 2.65 g cm^{-3}

The pH was analyzed by a glass combination pH electrode and a pH meter (Metler Toledo InLab®413 combination meter). Soil particle size analysis was performed according to the modified pipette method (Gavlak, et al., 2005). Total C and N analysis was performed at University of Maryland Department of Environmental Science and Technology Analytical Lab on LECO CN628 Elemental Analyzer (LECO Corp., St. Joseph, MI; Nelson and Sommers, 1996; Matejovic, 1993). Soil organic matter (SOM) (Loss on Ignition Method) and nutrient content by Mehlich3 extraction (P, K, Mg, Ca, Na, S) was measured at WayPoint Analytical, Inc (Richmond, VA).

Statistical analysis

All analyses were performed using SAS version 9.4 statistical software (SAS Institute, Cary, NC). The level of probability considered significant was p < 0.05, unless otherwise stated. All ANOVA tests were performed using Proc Mixed. To investigate differences between the residual NH₄-N and NO₃-N amounts (objective two), an ANOVA was performed for 0-30 cm, 30-90 cm, 90-150 cm, and 150-210 cm depth increments for all farms, for Coastal Plain farms, for Acidic rock farms, and for Calcareous rock farms, with N-type (NO₃-N or NH₄-N) as a fixed effect and farm as a random effect. To compare pools of inorganic N among soils formed from Coastal Plain, Acidic rock, and Calcareous rock parent materials (objective three), an ANOVA was performed for each 30 cm increment soil depth for the amount of NO₃-N, the amount of NH₄-N, and the NO₃-N percent of the total mineral N, with parent material group as the fixed effect. To investigate differences in inorganic N among soil depths for each parent

material group (objective three), an ANOVA was performed for each parent material group for the amount of NO₃-N, the amount of NH₄-N, and the NO₃-N percent of the total mineral N, with soil depth as a fixed effect and farm as a random effect. To compare pools of inorganic N following corn versus following soybean crops (objective four), for the farms with side-by-side corn and soybean fields, an ANOVA was performed for each 30 cm increment soil depth for the amount of NO₃-N and the amount of NH₄-N, with crop type (corn or soybean) as the fixed effect and farm as a random effect. A Pearson product-moment correlation was performed using Proc Corr to relate the soil NO₃-N, NH₄-N, and NO₃-N percent of the total mineral N to soil percentages of sand, clay, silt, total C and total N, and the C/N ratio.

Results

Total mineral N in the 0-210 cm profiles ranged from 87.4 to 515 kg N ha⁻¹ (Figure 3). Across the 29 sites, there was on average of 253 kg ha⁻¹ of mineral N in the upper 210 cm of soil. About 22% of the mineral N was located in the uppermost 30 cm of soil, while another 23% was in the 30 to 90 cm increment. The 90-150 and 150-210 cm increments contained 27% and 28%, respectively, of the profile mineral N (Table 2).

Of the average total mineral N, 115 kg N ha⁻¹ was NO₃-N and 138 kg N ha⁻¹ was NH₄-N. For all layers of Acidic and Calcareous sites and the upper layers of Coastal Plain sites, there were no differences between the amounts of NO₃-N and NH₄-N. For the Coastal Plain sites, the amount of NO₃-N was significantly lower than the amount of NH₄-N in the subsoil layers (90-150 cm and 150-210 cm) (Table 2).

The distribution of NO₃-N among soil depth layers followed different patterns for each of the parent material groups. For the Coastal Plain sites, soil NO₃-N was greater from 0-30 cm than all of the other 30 cm depth layers from 30-210 cm. For the Acidic sites, soil NO₃-N was greater in the surface soil layer (0-30 cm) and some deep soil layers (120-150 cm, 180-210 cm) than 30-60 cm and/or 60-90 cm layers. For the Calcareous sites, there were no differences in soil NO₃-N among soil depth layers. The distribution of NH₄-N among soil depth layers followed similar patterns for each parent material groups—the surface layer (0-30 cm) soil had significantly more NH₄-N than all deeper layers for Coastal Plain, Acidic, or Calcareous sites, with the exception of 120-150 cm for Acidic sites. The NO₃-N percent of the total mineral N was not different among soil depth layers for the Coastal Plain sites or Calcareous sites. For the Acidic sites, the NO₃-N percent of the total mineral N was lower for the 0-30 and 30-60 soil depth layers than the 30 cm increment soil depth layers from 90-210 cm (Figure 4).

There were differences among parent material groups for the amount of NO₃-N and the NO₃-N percent of the total mineral N for some soil depth layers, but there were no differences among parent material groups for the amount of NH₄-N at any soil depth layer. The Coastal Plain sites had lower soil NO₃-N levels than the Acidic sites at 90-120 cm and 120-150 cm depth layers, and than the Calcareous sites at 150-180 cm soil depth. The Coastal Plain sites also had lower NO₃-N percent of the total mineral N than the Acidic sites at 90-120 cm, 120-150 cm, and 150-180 cm soil depth layers, and than the Calcareous sites at 120-150 cm, 150-180 cm, and 180-210 cm soil depth layers (Figure 4).

We correlated soil percents of sand, clay, silt, total C and total N, and the C/N ratio with the pool sizes of soil NO₃-N and NH₄-N, and NO₃-N percent of the total mineral N in the profiles (Table 3; Table 4). The percent sand was negatively correlated to the NO₃-N concentration (p < 0.10) in the 0-30 cm, 90-150 cm, and 150-210 cm soil. In the topsoil layer (0-30 cm), the percent C and percent N were positively correlated (p < 0.05) to soil NO₃-N and to the NO₃-N percent of the total mineral N. In the 30-90 and 90-150 cm soil depths, we found a negative correlation (p < 0.1) between pH and NH₄-N content, and we found a positive correlation (p < 0.05) between pH and NO₃-N percent of the total mineral N.

There was significantly more soil NO₃-N in September following soybean than following corn in the soil depth increments of 30-60 cm, 120-150 cm, 150-180 cm, and 180-210 cm. The levels of soil NH₄-N did not differ between corn or soybean treatments, except in the 180-210 cm soil increment, in which soil NH₄-N following soybean was significantly higher than following corn (Figure 5).

Discussion

We expected surface layers to have higher mineral N, as surface soil layers have the most incorporated plant residues, fertilizer, roots and microbial activity. Soil NH₄-N was always higher on surface soil layers than deeper soil layers, and soil NO₃-N was higher on surface soil layers than deeper soil layers in some cases. The decomposition and mineralization of surface sources of organic C and N likely resulted in the positive correlations between topsoil percent C or N and the amount of soil NO₃-N or the NO₃-N percent of the total mineral N.

Coastal Plain sites also had less NO₃-N in all subsoil layers (30-210 cm deep) than the 0-30 layer, whereas the Acidic and Calcareous sites were more variable. Nitrate-N would be expected to leach more quickly through sandy soils, and we did find that percent sand was negatively correlated with the soil NO₃-N concentration (but had no relationship with NH₄-N concentration).

Across all farms, approximately half of the mineral N was in the NO₃-N form and half NH₄-N form. It is not uncommon, especially on manured soils, for NH₄-N concentrations to be as high or even higher than NO₃-N concentrations (Brandi-Dohrn, et al., 1997; Eghball, et al., 2004; Kristensen and Thorup-Kristensen, 2004b; Lacey and Armstrong, 2015; Sainju, et al., 2007). Greater NH₄-N levels could be attributed to ammonification exceeding nitrification due to higher soil water content or due to NH₄-N retention on clay particle cation exchange sites in the subsoil (Sainju, et al., 2007). Soil NO₃-N is assumed to be more transient than soil NH₄-N, in that soil NO₃-N is accumulating and leaching from the soil each year while soil NH₄-N is being retained for multiple years in the soil through cation exchange. However, we did not find a positive correlation between percent clay and NH₄-N amounts. This is likely because NH₄-N ions are occupying only a small fraction of the cation exchange sites, and therefore all of the soils have clay contents high enough to accumulate NH₄-N cations.

Ammonium-N levels did not vary among parent material types or between soybean and corn fields (except for at one depth), whereas NO₃-N levels varied among parent material types and between corn and soybean crops. Previous studies have found NO₃-N levels to be more dynamic than NH₄-N levels. Kristensen and Thorup-Kristensen (2004b) found that October residual NO₃-N (0-2.5 m profile) varied between crop

species, with sweet corn (*Zea mays* L. *Saccharata* Koern.) > carrot (*Daucus carota* L.) > white cabbage (*Brassica oleracea* L. convar. *Capitata*), whereas residual NH₄-N did not vary between the different species. From soil cores taken in various barley (*Hordeum vulgare* L.), fescue (*Festuca* L.), and alfalfa (*Medicago sativa* L.) cropping systems (samples 1 m deep, six to nine times per year), Bergstrom (1986) found NH₄-N did not vary much between treatments, staying between 11 and 13 kg N ha⁻¹, whereas NO₃-N ranged between 23 and 68 kg N ha⁻¹. On a silt loam soil and a loamy sand soil in Wisconsin, Bundy, et al. (1993) found that spring soil NO₃-N (0-90 cm) was higher following soybean in a corn/soybean rotation than following corn in a no-fertilizer continuous corn rotation, but there was no consistent effect of corn/soybean sequence on NH₄-N levels.

Soil inorganic N might be expected to increase following corn versus soybean cash crops since corn receives N fertilization, while soybean, a legume, does not usually receive N fertilization. However, we found higher levels of NO₃-N following soybean than following corn. Other previous studies have also found corn did not have higher residual soil NO₃-N levels than soybean following crop harvest (Jaynes, et al., 2001; Pantoja, et al., 2016; Rembon and MacKenzie, 1997). In Nebraska on a Sharpsburg silty clay loam soil, Kessavalou and Walters (1999) found that May soil residual NO₃-N (0-150 cm) was lower in a continuous corn system than following corn in the corn/soybean rotation system, even though it was fertilized more often (every year) and had 25% less N removed in corn yield than the corn in the corn/soybean rotation.

We hypothesize that in well-aerated surface soils, NH₄-N released during mineralization is rapidly converted to NO₃-N by nitrification, resulting in a high NO₃-N

percent of the total mineral N where immobilization has not removed the mineral N. This is most evident in comparing the soil mineral N after soybeans versus after corn. Mineral N may be higher following soybean than corn because the soil in a soybean crop is a high N environment with low C/N residues and high N root exudates. Microbial N immobilization, which would remove NO₃-N and NH₄-N from the soil solution, would be expected to be much lower with soybean residue than with corn residue. Green and Blackmer (1995) found higher N immobilization following corn, due to the larger amount of corn residue than soybean residue, which allowed N to be more available following soybean.

Concerning soil acidity, our findings were as expected. We expected that at the lowest pH levels (pH 4-5), nitrification (NH₄ transformed to NO₃) would be limited, leading to higher NH₄ amounts and a lower NO₃-N percent of the total mineral N. We also expected that at high pH levels, ammonium could be lost through ammonia volatilization (NH₄ transformed to NH₃), leading to lower NH₄ amounts and a higher NO₃-N percent of the total mineral N (Table 4).

Conclusions and practical applications

Across all sites, 57% (65 kg N ha⁻¹) of NO₃-N and 55% (138 kg N ha⁻¹) of total mineral N to 210 cm was located 90-210 cm deep. This large pool of deep soil mineral N remaining after growing corn and soybean poses an environmental risk as the N can leach from the system and pollute bodies of water. It also poses an economic risk if this N is lost to the farmer. On the other hand, if this N was recycled to the surface of the soil where it could provide a substantial amount of N to subsequent crops, it might allow farmers to reduce fertilizer applications.

The findings that soil NO₃-N was higher following soybean than following corn in much of the soil profile is important for management considerations. Residual soil N is often assumed to be a result of over applying N fertilizer (https://www.npr.org/sections/thesalt/2017/03/07/518841084/farmers-fight-environmental-regulations), and management practices and policies are primarily concerned with preventing fields that have had fertilizer applications from polluting water sources (Maryland Department of Agriculture, 2014). Legumes such as soybean are not typically fertilized with N, yet our data shows they can leave even more residual N in the soil profile and could pose an even greater risk for water pollution than fertilized crops such as corn.

The vertical location of the N is important. Many studies that report effects of cover crops on soil N (Chu, et al., 2017; Ebelhar, et al., 1984; Kuo and Jellum, 2002; Ladoni, et al., 2015; Ruffo, et al., 2004; Sainju, et al., 2006) or other cropping practices on soil N (Anderson and Peterson, 1973; Poudel, et al., 2002; Rice, et al., 1986; Scalise, et al., 2015) after taking 15-30 cm deep soil cores may miss important N patterns in deeper soil layers. Shallow soil sampling may be due to the difficulty in obtaining deeper soil cores and the misconception that N deeper in the profile would be an insignificant amount and/or beyond the reach of roots. However, the deeper N (1-2 meters deep) is most at-risk for leaching from the system. Therefore, practices such as incorporating deep-rooted cover cropping systems into crop rotations should be encouraged in order to scavenge deep soil N before it is lost from the system.

Table 1. Site descriptions for soil core transect sites, indicating crop, manure, and tillage history, and soil descriptions. Physiographic regions were determined according to Polsky, et al. (2000). Soil series and phase were determined from Web Soil Survey (WSS) data from USDA NRCS (https://websoilsurvey.sc.egov.usda.gov/App/HomePage.htm); soil sample texture was compared to the official soil series descriptions from USDA NRCS (https://www.nrcs.usda.gov/wps/portal/nrcs/detail/soils/home/?cid=nrcs142p2_053587) to ensure soil samples correlated to the mapping units. Parent material classification based on soil series description and verified with observed soil characteristics (e.g., pH and texture).

Site Name	Physiographic region/Parent material classification	Site Map No.	Sampling date	Most recent or current vegetation at sampling point	Crop rotation history	Manure history	Tillage history	Mapped soil series and phase
Caroline I	Coastal Plain	24	29 Aug 2014	Standing corn	Corn/soybean rotation previous 10 years	Occasio nal	2013 no-till, 2012 sludge incorporated, 2005-2011 no- till, 2004 vertical tillage	Ingleside sandy loam, 2-5% slopes; Hambrook loam, 0- 2% slopes
Caroline II	Coastal Plain	20	28 Aug 2014	Standing corn	NA ¹	NA	NA	Unicorn-Sassafras loams, 0-2% slopes
Carroll I	Piedmont/ Calcareous rocks	10	1 Sep 2014	Perennial grass (alley between tomatoes)	NA	NA	NA	Conestoga silt loam, 3-8% slopes
Dorchester IA	Coastal Plain	22	29 Aug 2014	Standing corn	NA	NA	NA	Nassawango silt loam, 0-2% slopes
Dorchester IB	Coastal Plain	23	17 Aug 2015	Recently harvested wheat	NA	NA	NA	Fallsington sandy loams, 0-2% slopes, Northern Tidewater Area
Franklin I	Ridge and Valley/ Acidic rocks	4	19 Sep 2014	Recently harvested corn	Corn, small gran silage, alfalfa	Regular	No-till	Murrill gravelly loam, 8-15% slopes

Franklin IIB	Ridge and Valley/ Calcareous rocks	5	25 Sep 2015	Recently harvested corn	Corn, small grain silage	Regular	Mostly no-till; Occasional tillage	Hagerstown silt loam, 0-3% slopes
Frederick I	Piedmont/ Acidic rocks	1	6 Sep 2014	Recently harvested corn	NA	Regular	NA	Penn loam, 3-8% slopes
Frederick II	Piedmont/ Calcareous rocks	2	6 Sep 2014	Recently harvested corn	unknown	Regular	unknown	Hagerstown loam, 3-8% slopes
Frederick IV	Piedmont/ Calcareous rocks	3	28 Aug 2015	Recently harvested corn	Double crop corn/triticale for > 5 years	Regular manure applicati ons spring and fall for past 10 years	Subsoiled in 2014, disked once yr ⁻¹ until 2016	Duffield-Ryder silt loams, 0-3% slopes
Harford I	Piedmont/ Acidic rocks	11	27 Aug 2014	Recently harvested corn	NA	Regular	NA	Chester gravelly silt loam, 3-8% slopes, moderately eroded
Howard IA	Piedmont/ Calcareous rocks	12	5 Sep 2014	Recently harvested corn	2014 corn silage, 2013 corn, 2012 forage sorghum, 2011 sweet corn, 2010 sweet corn	Occasio nal	No-till	Hatboro-Codorus silt loams, 0-3 % slopes
Howard IB	Piedmont/ Acidic rocks	13	26 Aug 2015	Recently harvested corn	2015 corn silage, 2014 soybean, 2013 corn grain (rye cover crop), 2012 corn grain, 2011 corn grain, 2010 corn grain	No manure applicati ons past 20+ years	No-till	Glenelg loam, 3- 8% slopes; Manor loam, 8-15% slopes
Howard IC	Piedmont/ Acidic rocks	14	29 Oct 2016	Standing corn	2010-2015 Timothy hay	2 manure applicati	No-till	Gladstone loam, 3 to 8 percent slopes

						ons past 10 years		
Howard ID	Piedmont/ Acidic rocks	15	29 Oct 2016	Standing soybean	2015 corn silage (rye cover crop), 2014 corn, 2013 sorghum, 2012 corn (rye cover crop), 2011 soybean, 2010 corn (rye cover crop)	1 manure applicati ons past 10 years	No-till	Gladstone loam, 3 to 8 percent slopes
Kent I	Coastal Plain	21	28 Aug 2014	Standing corn	NA	NA	NA	Butlertown- Mattapex silt loams, 2-5% slopes, moderately eroded
Kent II	Coastal Plain	25	11 Sep 2015	Recently harvested corn	NA	NA	NA	Mattapex fine sandy loam, 0-2% slopes
Kent IIB	Coastal Plain	26	25 Sep 2016	Recently harvested corn	NA	NA	NA	Mattapex fine sandy loam, 0 to 2 percent slopes
Kent IIC	Coastal Plain	27	25 Sep 2016	Standing soybean	NA	NA	NA	Mattapex fine sandy loam, 0 to 2 percent slopes
Kent IID	Coastal Plain	28	24 Sep 2016	Recently harvested corn	NA	NA	NA	Matapeake silt loam, 0 to 2 percent slopes
Kent IIE	Coastal Plain	29	24 Sep 2016	Standing soybean	NA	NA	NA	Matapeake silt loam, 0 to 2 percent slopes
Lancaster IA	Piedmont/ Acidic rocks	6	2 Sep 2014	Recently harvested wheat followed by cover crop	2013 pumpkin, 2012 corn, 2011 corn, 2010 soybean	Occasio nal	No-till past 5+ years	Glenelg silt loam, 8-15% slopes

				mix (60 cm tall)				
Lancaster IB	Piedmont/ Acidic rocks	7	12 Sep 2015	Recently harvested corn	2014 pumpkin, 2013 corn, 2012 soybean, 2011 corn	Occasio nal	No-till past 5+ years	Glenelg silt loam, 3-8% slopes
Lancaster V	Piedmont/ Calcareous rocks	8	12 Sep 2015	Recent tobacco	Corn silage, forage rye, tobacco, alfalfa rotation	Regular	Mostly no-till corn, some no-till tobacco	Duffield silt loam, 3-8% slopes
Prince George's I	Coastal Plain	18	25 Aug 2014	Recently harvested corn	2013 soybean, 2012 corn, 2009-2011 mixed grass hay with < 25% legumes	No manure ever applied	No-till past 5+ years	Collington-Wist complex, 0-2% slopes
Prince Georges IIIA	Coastal Plain	16	15 Oct 2016	Standing corn	2015 wheat double crop soybean, 2014 corn, 2013 wheat double crop soybean, 2012 soybean, 2011 corn	None	2011-2013 notill; fall 2013 chisel plow prior to wheat, 2015-2016 no-till	Russett-Christiana complex, 0 to 2 percent slopes
Prince Georges IIIB	Coastal Plain	17	15 Oct 2016	Recently harvested soybean	2015 wheat double crop soybean, 2014 soybean, 2013 wheat double crop soybean, 2012 soybean, 2011 corn	None	2011-2013 notill; fall 2013 chisel plow prior to wheat, 2015-2016 no-till	Russett-Christiana complex, 0 to 2 percent slopes
St. Mary's I	Coastal Plain	19	20 Aug 2014	Pasture	2011-2014 Sudex in summer with rye/clover in winter, 2010 soybean, 2009 corn	2011- 2014 no manure; 2004- 2010 regular applicati ons poultry litter	2011-2014 notill, 2010 and before likely vertical tillage	Sassafras loam, 0- 2% slopes

York I	Piedmont/ Acidic rocks	9	20 Sep 2014	Recently harvested corn	Unknown	Regular	No-till	Chester silt loam, 3-8% slopes		
¹ NA indicates information not available										

Table 2. Soil NO₃-N, NH₄-N, and mineral N (NO₃-N + NH₄-N) (kg N ha⁻¹) for 0-30 cm, 30-90 cm, 90-150 cm, 150-210 cm, and 0-210 cm, and the percent of total mineral N found in each soil depth increment. Values are average of all sites (N=29), Coastal plain sediments sites (N=14), Calcareous rocks sites (N=6), and Acidic rocks sites (N=9). Within a depth increment, values followed by the same lower case letter do not differ significantly.

significantly.				
	Depth			
Site	increment	NO ₃ -N	NH4-N	Mineral N
	cm	kg N ha ⁻¹ (% of	0-210 cm N for dep	oth increment)
	0-30	24.9 (22%) a	31.3 (23%) a	56.3 (22%)
	30-90	25.2 (22%) a	33.6 (24%) a	58.7 (23%)
All site	90-150	30.8 (27%) a	37.0 (27%) a	67.7 (27%)
	150-210	33.9 (30%) a	36.0 (26%) a	69.9 (28%)
	0-210	115 a	138 a	253
	0-30	23.9 (27%) a	30.0 (22%) a	53.9 (24%)
Coastal	30-90	23.8 (27%) a	33.5 (24%) a	57.3 (25%)
Plain	90-150	20.0 (23%) a	35.7 (26%) b	55.7 (25%)
sediments	150-210	20.7 (23%) a	38.1 (28%) b	58.8 (26%)
	0-210	88.4 a	137 b	226
	0-30	24.1 (18%) a	35.9 (23%) a	60.0 (21%)
	30-90	25.2 (19%) a	36.2 (24%) a	61.4 (21%)
Acidic rocks	90-150	44.5 (33%) a	43.0 (28%) a	87.5 (30%)
	150-210	42.4 (31%) a	38.1 (25%) a	80.5 (28%)
	0-210	136 a	153 a	289
	0-30	28.5 (20%) a	27.8 (24%) a	56.3 (22%)
Calcareous	30-90	28.1 (19%) a	29.9 (26%) a	58.0 (22%)
rocks	90-150	35.3 (25%) a	30.9 (27%) a	66.3 (25%)
TUCKS	150-210	52.2 (36%) a	28.0 (24%) a	80.2 (31%)
	0-210	144 a	117 a	261

Table 3. Twenty-nine farm mean, standard deviation (SD), and range values of soil NO_3 -N (kg N ha⁻¹), NH₄-N (kg N ha⁻¹), and NO₃-N percent of the total mineral N (NO₃-N + NH₄-N), pH, percent sand, clay, and silt, percent total C, percent total N, and C/N ratio. Soil divided into increments of 0-30 cm, 30-90 cm, 90-150 cm, and 150-210 cm. The percent total N and C/N ratio calculated for 0-30 cm increment only, due to many below detection limit (BDL) N levels in deeper layers.

		0-30 cm	n	3	30-90c	m		90-150)cm		150-210	em
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
NO ₃ -N (kg ha ⁻¹)	24.9	20.6	3.15 – 92.0	25.2	17.6	2.22 – 84.4	30.8	19.7	5.40 – 70.3	33.9	30.2	1.77 - 153
NH ₄ -N (kg ha ⁻¹)	31.3	14.7	11.9 – 78.9	33.6	21.0	12.0 – 96.8	37.0	25.3	10.1 - 110	36.0	26.6	9.63 - 115
NO ₃ -N % of min N	0.404	0.146	0.153- 0.757	0.419	0.148	0.105- 0.691	0.458	0.145	0.163- 0.752	0.474	0.172	0.0810- 0.818
pН	6.14	0.479	5.14 – 7.26	5.87	0.725	4.51 – 7.40	5.56	0.69	4.09 – 7.02	5.44	0.76	4.04 – 7.40
Sand (%)	37.9	13.5	10.5 – 58.1	42.0	15.2	13.9 – 70.6	55.6	22.5	18.7 – 90.1	60.2	24.9	13.4 – 93.8
Clay (%)	16.7	5.10	7.71 – 25.2	23.8	7.99	10.8 – 44.2	20.0	13.4	3.63 – 53.9	17.1	14.3	2.31 – 55.4
Silt (%)	45.4	11.2	27.9 – 72.3	34.2	11.4	14.6 – 62.2	24.4	14.2	3.71 – 53.1	22.6	14.3	3.78 – 47.2
C (%)	0.897	0.342	0.351 – 1.66	0.271	0.207	0.133 – 1.23	0.15	0.11	0.0472 – 0.651	0.12	0.16	0.0303 - 0.863
N (%)	0.0919	0.0361	0.0466 – 0.170	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
C:N	9.80	1.63	5.76 – 14.1	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL

Table 4. Correlations coefficient (r) and significance (p-value) for correlations between soil NO_3 -N (kg N ha⁻¹), NH_4 -N (kg N ha⁻¹), and NO_3 -N percent of the total mineral N (NO_3 -N + NH_4 -N) with soil percent sand, clay, silt, total C, total N, C/N ratio, and pH. Data for 29 farms analyzed by profile increments of 0-30 cm, 30-90 cm, 90-150 cm, and 150-210 cm. The percent total N and C/N ratio correlated for 0-30 cm increment only, due to N levels below detection limit.

due to iv levels bei			Se	oil textur	e	Perce	ent C a	nd N	TT
			% Sand	% Clay	% Silt	% C	% N	C/N	pН
	0.20	r	-0.38	-0.020	0.46	0.42	0.38	-0.040	0.12
	0-30 cm	p-value	0.044	0.92	0.012	0.023	0.041	0.84	0.52
	30-90 cm	r	0.010	-0.067	0.034	0.13			0.086
NO. N	30-90 CIII	p-value	0.96	0.729	0.86	0.51			0.66
NO ₃ -N	90-150 cm	r	-0.34	0.23	0.33	0.17			-0.078
	90-150 CIII	p-value	0.068	0.23	0.08	0.38	•	•	0.69
	150-210 cm	r	-0.40	0.40	0.30	0.026			-0.19
	150-210 CIII	p-value	0.03	0.03	0.11	0.89			0.33
	0-30 cm	r	-0.27	0.21	0.23	0.11	0.11	-0.11	-0.25
	0-30 CIII	p-value	0.16	0.28	0.23	0.58	0.58	0.58	0.19
	30-90 cm	r	-0.29	-0.03	0.41	0.062			-0.36
NH4-N	30-90 CIII	p-value	0.13	0.87	0.028	0.75	•	•	0.058
IN I 14-IN	90-150 cm	r	-0.097	-0.032	0.18	0.061	•		-0.32
	90-150 CIII	p-value	0.62	0.87	0.34	0.754	•	•	0.087
	150-210 cm	r	-0.065	-0.020	0.13	-0.074			-0.21
	150-210 CIII	p-value	0.74	0.92	0.49	0.72	•	•	0.28
	0-30 cm	r	-0.22	-0.081	0.31	0.38	0.38	-0.13	0.18
	0-30 Cm	p-value	0.24	0.68	0.11	0.040	0.044	0.50	0.35
	30-90 cm	r	0.18	-0.088	-0.18	0.11			0.43
NO ₃ -N % of min N	30-70 CIII	p-value	0.35	0.65	0.36	0.58	•		0.019
14O3-14 /0 OI IIIII IV	90-150 cm	r	-0.20	0.094	0.23	0.12	•		0.38
	90-130 CIII	p-value	0.30	0.63	0.24	0.52			0.041
	150-210 cm	r	-0.12	0.044	0.16	0.13	•		0.23
	130-210 CIII	p-value	0.54	0.82	0.40	0.51			0.23

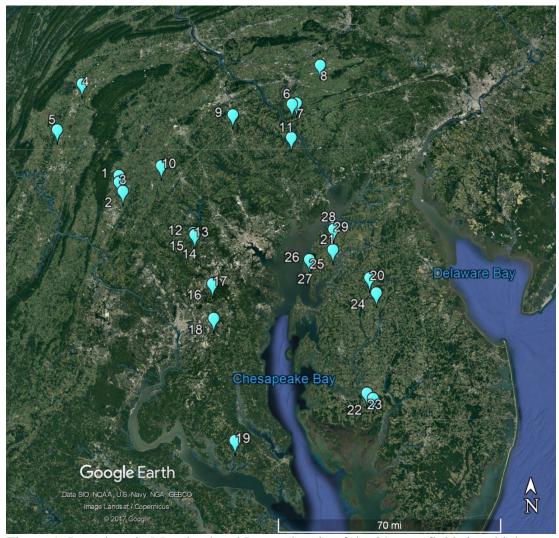


Figure 1. Locations in Maryland and Pennsylvania of the 29 crop fields in which a transect of 0-210 cm deep soil cores were taken.

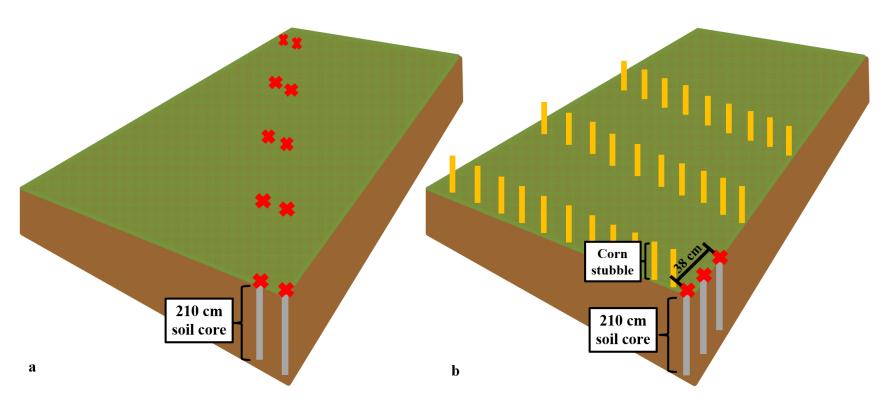


Figure 2. Deep soil core placement scheme for (a) 2014 showing placement of all five sets of cores per field, and (b) 2015 showing positions for one of the four sets of cores per field.

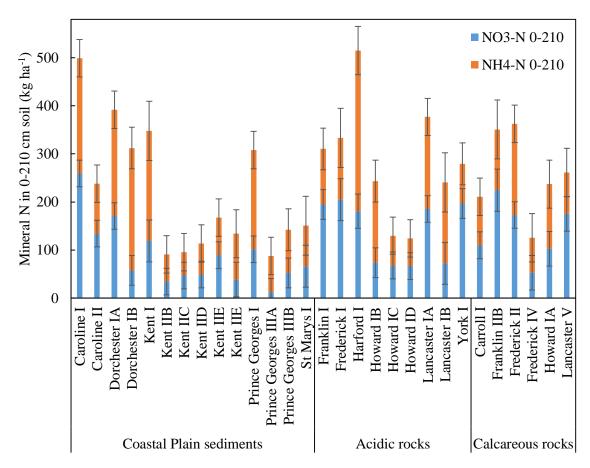


Figure 3. Twenty-nine farm 0-210 cm NO_3 -N (kg N ha⁻¹) and NH₄-N (kg N ha⁻¹). Error bars show standard error (SE) of mean. Sites Dorchester IB and Lancaster IB total is for 0-180 cm only.

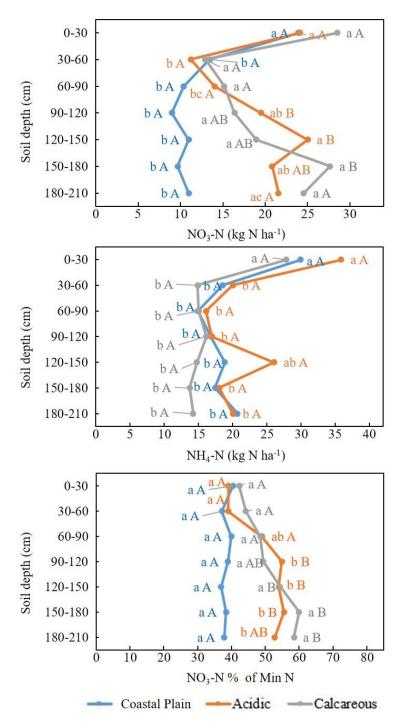


Figure 4. Amount of NO₃-N and NH₄-N (kg N soil layer⁻¹ ha⁻¹) and NO₃-N percent of the total mineral N (NO₃-N + NH₄-N) of each 30 cm depth increment for sites with Coastal Plain sediments, Acidic rocks, and Calcareous rocks parent materials. Different lowercase letters indicate significant differences among depths within each parent material group. Different uppercase letters indicate significant differences among parent material groups within each depth.

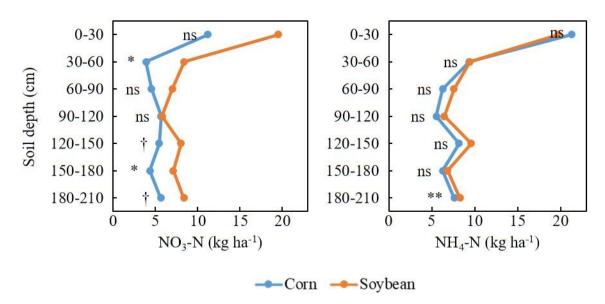


Figure 5. NO₃-N and NH₄-N (kg N soil layer⁻¹ ha⁻¹) in four pairs of adjacent corn and soybean fields. The symbols **, *, †, ns indicate p < 0.01, 0.05, 0.1, and not significant.

Appendix 1. Transect site soil characteristics

Table 5. Study site soil pH, percent sand, percent clay, soil texture, percent C, and percent N for each 15 or 30 cm soil depth increment (0-210 cm), and percent soil organic matter (SOM), P, K, Mg, Ca, and S (mg kg⁻¹) for the upper 30 cm of soil from each site. Each record is the average of two to three composited soil cores from two areas in the field. Data from Dorchester IB 180-210 cm, Lancaster IB 180-210 cm, and St Marys I 195-210 cm is from a single point of a field. Values not determined indicated as nd. Values below detection limit indicated as BDL.

Site	Depth	pH ¹	Texture	Sand ²	Clay ²	\mathbb{C}^3	N^3	SOM ⁴	\mathbf{P}^4	K ⁴	Mg^4	Ca ⁴	S^4
	cm					%					ppm		
	0-15	5.7	Sandy loam	59.7	7.3	0.711	0.066	1.55	51.5	59	45	498	6
	15-30	5.9	loam	48.7	13.7	0.240	0.027	1.1	5	70	66	474	1
	30-45	nd	nd	nd	nd	0.187	BDL						
	45-60	5.6	Sandy loam	62.1	15.1	0.155	BDL						
	60-75	nd	nd	nd	nd	0.121	BDL						
	75-90	5.2	Sandy loam	65.8	17.7	0.071	BDL						
Canalina I	90-105	nd	nd	nd	nd	0.058	BDL						
Caroline I	105-120	5.2	Sandy loam	73.5	19.0	0.053	BDL						
	120-135	nd	nd	nd	nd	0.096	BDL						
	135-150	5.2	Sandy loam	76.5	18.3	0.067	BDL						
	150-165	nd	nd	nd	nd	0.054	BDL						
	165-180	4.7	Sandy clay	73.9	21.2	0.053	BDL						
	180-195	nd	nd	nd	nd	0.035	BDL						
	195-210	4.7	Sandy loam	73.7	18.4	0.087	BDL						
	0-15	6.3	Sandy loam	56.2	8.8	0.765	0.070	1.9	149	113	64.5	783	9.5
	15-30	6.3	Sandy loam	52.7	13.1	0.418	0.037	1.4	61.5	101	73	597	4.5
Caroline II	30-45	nd	nd	nd	nd	0.303	0.031						
	45-60	6.2	Sandy loam	59.1	17.5	0.246	BDL						
	60-75	nd	nd	nd	nd	0.169	BDL						

	75-90	6.3	Sandy loam	82.1	12.2	0.057	BDL						
	90-105	nd	nd	nd	nd	0.083	BDL						
	105-120	6.2	Loamy fine	85.3	8.7	0.053	BDL						
	120-135	nd	nd	nd	nd	0.132	BDL						
	135-150	6.2	Loamy fine	81.8	9.4	0.068	BDL						
	150-165	nd	nd	nd	nd	0.064	BDL						
	165-180	6.2	Loamy fine	87.6	5.8	0.070	BDL						
	180-195	nd	nd	nd	nd	0.045	BDL						
	195-210	6.3	Fine sand	87.9	5.5	0.054	BDL						
	0-15	6.0	Silt loam	22.7	10.6	1.246	0.122	2.85	91	159	136	757	15
	15-30	5.8	Silt loam	16.4	17.6	0.614	0.066	1.6	32	96	92.5	558	9
	30-45	nd	nd	nd	nd	0.356	0.053						
	45-60	5.6	Silt loam	13.0	24.0	0.193	0.037						
	60-75	nd	nd	nd	nd	0.142	0.030						
	75-90	5.2	loam	44.5	21.1	0.105	0.026						
Dorchester IA	90-105	nd	nd	nd	nd	0.098	0.024						
Dorchester IA	105-120	5.3	Sandy loam	67.4	16.5	0.054	BDL						
	120-135	nd	nd	nd	nd	0.308	0.042						
	135-150	5.3	Sandy loam	76.5	9.7	0.153	BDL						
	150-165	nd	nd	nd	nd	0.084	BDL						
	165-180	5.3	Loamy fine	81.8	10.4	0.090	BDL						
	180-195	nd	nd	nd	nd	0.050	BDL						
	195-210	5.3	Loamy fine	81.2	10.0	0.052	BDL						
	0-30	6.0	Silt loam	34.9	12.8	0.805	0.071	2.25	47.5	43	75.5	643	20
	30-60	5.2	loam	35.4	20.0	0.170	BDL						
Dorchester IB	60-90	4.5	Silt loam	20.1	19.6	0.160	BDL						
	90-120	4.8	loam	45.7	17.6	0.150	0.025						
	120-150	4.9	Sandy loam	59.2	15.3	0.146	BDL						

	150-180	5.1	Sandy loam	62.9	15.2	0.130	BDL						
	180-210	6.0	loam	27.1	26.9	0.181	0.03						
	0-15	5.3	Silt loam	11.2	20.3	0.434	0.061	1.65	7.5	53.5	188	791	44.5
	15-30	5.0	Silt loam	9.9	22.3	0.267	0.044	1.45	2.5	45.5	197	692	71
	30-45	nd	nd	nd	nd	0.251	0.040						
	45-60	4.8	Silt loam	24.4	21.3	0.168	0.029						
	60-75	nd	nd	nd	nd	0.115	BDL						
	75-90	4.8	loam	37.9	20.4	0.316	BDL						
Kent I	90-105	nd	nd	nd	nd	0.187	BDL						
Kelit I	105-120	4.8	Sandy loam	59.8	15.6	0.097	BDL						
	120-135	nd	nd	nd	nd	0.206	0.023						
	135-150	5.0	Loamy fine	79.2	6.6	0.141	BDL						
	150-165	nd	nd	nd	nd	0.084	BDL						
	165-180	4.9	Loamy fine	85.7	4.1	0.075	BDL						
	180-195	nd	nd	nd	nd	0.070	BDL						
	195-210	4.7	Loamy fine	86.0	3.3	0.059	BDL						
	0-30	6.0	loam	51.6	7.7	0.635	0.051	2.65	27.5	72	58	495	2
	30-60	6.3	loam	45.7	16.1	0.247	0.030						
	60-90	6.3	Loamy fine	81.1	5.6	0.097	BDL						
Kent II	90-120	6.2	Fine sand	92.9	3.6	0.037	BDL						
	120-150	6.2	Fine sand	87.2	3.7	0.065	BDL						
	150-180	6.3	Fine sand	94.8	1.5	0.031	BDL						
	180-210	5.6	Fine sand	92.8	3.1	0.037	BDL						
	0-15	6.0	loam	45.8	8.2	1.015	0.101	2.1	40	62	98	519	3
	15-30	5.7	loam	44.5	10.1	0.509	0.056	1.45	21.5	39.5	48.5	414	2
Kent IIB	30-45	nd	nd	nd	nd	0.292	0.036						
	45-60	5.9	loam	36.3	17.9	0.222	0.038						
	60-75	nd	nd	nd	nd	0.167	0.033						

	75-90	5.8	loam	49.3	17.7	0.120	0.027						
	90-105	nd	nd	nd	nd	0.067	0.022						
	105-120	5.6	Loamy fine	85.0	5.6	0.052	BDL						
	120-135	nd	nd	nd	nd	0.268	BDL						
	135-150	5.1	Loamy fine	84.0	3.5	0.052	BDL						
	150-165	nd	nd	nd	nd	0.050	BDL						
	165-180	5.3	Fine sand	91.4	3.7	0.038	BDL						
	180-195	nd	nd	nd	nd	0.031	BDL						
	195-210	4.8	Fine sand	92.6	2.7	0.035	BDL						
	0-15	6.2	loam	50.7	8.4	0.818	0.083	1.85	65.5	81	103	625	7
	15-30	6.2	loam	46.4	11.6	0.401	0.042	1.3	25.5	68.5	67.5	541	7.5
	30-45	nd	nd	nd	nd	0.281	0.045						
	45-60	6.1	loam	47.2	17.9	0.222	0.038						
	60-75	nd	nd	nd	nd	0.189	0.031						
	75-90	6.0	Sandy loam	58.2	14.2	0.104	0.022						
Kent IIC	90-105	nd	nd	nd	nd	0.082	BDL						
Kent IIC	105-120	6.0	Sandy loam	72.4	9.9	0.078	BDL						
	120-135	nd	nd	nd	nd	0.122	BDL						
	135-150	5.9	Sandy loam	71.4	7.5	0.061	BDL						
	150-165	nd	nd	nd	nd	0.056	BDL						
	165-180	5.7	Loamy fine	83.2	5.2	0.046	BDL						
	180-195	nd	nd	nd	nd	0.034	BDL						
	195-210	5.5	Loamy fine	86.9	5.9	0.045	BDL						
	0-15	6.1	Silt loam	36.5	12.8	0.984	0.098	2.2	49.5	177	101	551	6
	15-30	6.3	Silt loam	28.4	16.3	0.528	0.057	1.75	18.5	76	124	507	8
Kent IID	30-45	nd	nd	nd	nd	0.288	0.039						
	45-60	5.6	loam	43.2	21.1	0.200	0.029						
	60-75	nd	nd	nd	nd	0.110	BDL						

	75-90	5.5	Sandy loam	81.3	11.4	0.051	BDL						
	90-105	nd	nd	nd	nd	0.052	BDL						
	105-120	5.4	Fine sand	89.4	6.1	0.040	BDL						
	120-135	nd	nd	nd	nd	0.060	BDL						
	135-150	5.1	Fine sand	90.7	6.4	0.037	BDL						
	150-165	nd	nd	nd	nd	0.050	BDL						
	165-180	5.0	Loamy fine	87.9	7.4	0.050	BDL						
	180-195	nd	nd	nd	nd	0.042	BDL						
	195-210	5.0	Loamy fine	84.7	7.9	0.047	BDL						
	0-15	6.3	Silt loam	32.8	14.8	0.848	0.084	2.2	46	113	144	633	9
	15-30	6.2	Silt loam	32.8	16.7	0.582	0.063	1.9	23.5	94	142	607	5
	30-45	nd	nd	nd	nd	0.284	0.039						
	45-60	6.1	loam	46.5	19.1	0.242	BDL						
	60-75	nd	nd	nd	nd	0.296	BDL						
	75-90	6.1	Sandy loam	72.7	10.2	0.107	BDL						
Kent IIE	90-105	nd	nd	nd	nd	0.084	BDL						
Kent HE	105-120	6.1	Loamy fine	86.3	6.8	0.047	BDL						
	120-135	nd	nd	nd	nd	0.077	BDL						
	135-150	6.0	Loamy fine	86.4	7.7	0.032	BDL						
	150-165	nd	nd	nd	nd	0.026	BDL						
	165-180	5.9	Fine sand	88.7	6.3	0.026	BDL						
	180-195	nd	nd	nd	nd	0.032	BDL						
	195-210	6.1	Sandy loam	79.5	11.9	0.037	BDL						
	0-15	5.2	Sandy loam	59.7	11.9	1.416	0.128	2.9	61	144	165	633	14
	15-30	5.2	Sandy loam	56.5	16.0	0.657	0.068	1.85	42	112	152	643	9.5
Prince Georges I	30-45	nd	nd	nd	nd	0.550	0.063						
	45-60	4.9	Clay loam	43.4	30.6	0.357	0.052						
	60-75	nd	nd	nd	nd	0.193	0.037						

	75-90	4.6	Sandy clay	55.7	23.9	0.150	0.034						
	90-105	nd	nd	nd	nd	0.151	0.033						
	105-120	4.5	Sandy clay	69.6	20.0	0.187	0.031						
	120-135	nd	nd	nd	nd	0.353	0.044						
	135-150	4.5	Sandy clay	69.6	20.1	0.120	0.028						
	150-165	nd	nd	nd	nd	0.103	0.025						
	165-180	4.4	Sandy loam	78.3	14.9	0.103	0.023						
	180-195	nd	nd	nd	nd	0.091	BDL						
	195-210	4.4	Sandy loam	77.6	13.6	0.104	0.022						
	0-15	6.1	Sandy loam	58.0	9.1	1.038	0.073	2.35	39.5	35.5	89.5	777	4
	15-30	5.5	loam	52.0	13.5	0.462	0.033	1.3	7.5	27	52	430	24.5
	30-45	nd	nd	nd	nd	0.211	0.029						
	45-60	4.7	loam	46.2	22.9	0.146	0.022						
	60-75	nd	nd	nd	nd	0.146	0.026						
	75-90	4.3	Clay loam	34.1	39.9	0.094	0.029						
Drings Coorges III A	90-105	nd	nd	nd	nd	0.092	0.032						
Prince Georges IIIA	105-120	4.2	clay	20.6	50.1	0.084	0.033						
	120-135	nd	nd	nd	nd	0.100	0.032						
	135-150	4.0	clay	16.9	54.5	0.085	0.031						
	150-165	nd	nd	nd	nd	0.096	0.032						
	165-180	4.0	clay	12.2	55.7	0.064	0.030						
	180-195	nd	nd	nd	nd	0.075	0.029						
	195-210	4.0	clay	14.6	49.9	0.067	0.031						
	0-15	5.8	Sandy loam	54.8	8.8	1.169	0.099	2.5	21.5	41	57	548	2
	15-30	5.5	loam	47.3	15.6	0.433	0.041	1.4	5.5	24	44.5	425	11.5
Prince Georges IIIB	30-45	nd	nd	nd	nd	0.215	BDL						
	45-60	4.6	Clay loam	43.3	32.3	0.120	BDL						
	60-75	nd	nd	nd	nd	0.176	BDL						

	75-90	4.6	Sandy clay	46.1	29.2	0.093	BDL						
	90-105	nd	nd	nd	nd	0.083	BDL						
	105-120	4.4	Sandy clay	48.3	29.9	0.072	BDL						
	120-135	nd	nd	nd	nd	0.120	BDL						
	135-150	4.5	Sandy clay	51.6	26.7	0.077	BDL						
	150-165	nd	nd	nd	nd	0.179	BDL						
	165-180	4.5	Sandy clay	47.2	25.8	0.083	BDL						
	180-195	nd	nd	nd	nd	0.089	BDL						
	195-210	4.4	clay	22.3	42.0	0.098	0.038						
	0-15	6.2	loam	41.9	12.4	0.862	0.082	2.1	149	83	75	664	10
	15-30	5.3	loam	33.0	20.7	0.548	0.062	1.8	40	62.5	84	460	5
	30-45	nd	nd	nd	nd	0.403	0.056						
	45-60	5.7	loam	45.6	23.3	0.300	0.043						
	60-75	nd	nd	nd	nd	0.278	BDL						
	75-90	5.3	Sandy loam	72.4	13.5	0.175	BDL						
St Marys I	90-105	nd	nd	nd	nd	0.101	BDL						
St Wai ys 1	105-120	5.5	Loamy fine	82.4	12.2	0.087	BDL						
	120-135	nd	nd	nd	nd	0.204	BDL						
	135-150	5.4	Loamy fine	88.7	7.6	0.098	BDL						
	150-165	nd	nd	nd	nd	0.063	BDL						
	165-180	5.5	Fine sand	92.0	4.4	0.054	BDL						
	180-190	nd	nd	nd	nd	0.042	BDL						
	195-210	5.1	Fine sand	92.7	3.3	0.041	BDL						
	0-15	5.5	loam	45.9	18.9	0.943	0.092	2.35	19	69	127	777	18.5
	15-30	5.9	loam	46.5	22.4	0.458	0.051	1.65	9.5	56	122	718	15.5
Franklin I	30-45	nd	nd	nd	nd	0.309	0.037						
	45-60	5.8	Clay loam	39.0	32.7	0.393	0.046						
	60-75	nd	nd	nd	nd	0.183	0.028						

	75-90	6.5	clay	29.3	40.6	0.153	0.028						
	90-105	nd	nd	nd	nd	0.232	0.034						
	105-120	5.8	clay	29.2	40.5	0.177	BDL						
	120-135	nd	nd	nd	nd	0.211	0.031						
	135-150	4.8	Clay loam	26.7	27.9	0.220	0.031						
	150-165	nd	nd	nd	nd	0.202	0.032						
	165-180	4.9	Clay loam	21.7	29.4	0.118	0.026						
	180-195	nd	nd	nd	nd	0.131	0.027						
	195-210	4.9	loam	28.3	26.2	0.152	0.025						
	0-15	6.4	loam	38.5	16.5	1.097	0.118	2.75	80	71.5	125	948	12
	15-30	6.2	loam	41.1	17.4	0.536	0.060	1.85	24.5	48	101	929	12.5
	30-45	nd	loam	nd	nd	0.319	0.046						
	45-60	6.2	Clay loam	36.3	28.6	0.224	0.036						
	60-75	nd	nd	nd	nd	0.150	0.033						
	75-90	5.2	Clay loam	32.0	30.9	0.119	BDL						
Frederick I	90-105	nd	nd	nd	nd	0.130	0.027						
riedelick i	105-120	4.9	loam	42.7	24.1	0.131	0.030						
	120-135	nd	nd	nd	nd	0.183	0.034						
	135-150	4.8	loam	50.1	11.3	0.082	BDL						
	150-165	nd	nd	nd	nd	0.077	BDL						
	165-180	4.7	loam	47.2	11.4	0.064	BDL						
	180-195	nd	nd	nd	nd	0.081	0.023						
	195-210	4.9	loam	49.8	10.4	0.082	BDL						
	0-15	6.1	Silt loam	26.6	19.5	1.738	0.159	4.3	17	95	133	728	11
	15-30	5.7	loam	24.9	25.6	0.760	0.078	2.3	4	59	99	543	29
Harford I	30-45	nd	nd	nd	nd	0.433	0.051						
	45-60	5.3	loam	29.5	23.3	0.204	0.032						
	60-75	nd	nd	nd	nd	0.157	0.024						

	75-90	5.1	loam	38.5	16.9	0.107	BDL						
	90-105	nd	nd	nd	nd	0.083	BDL						
	105-120	5.2	loam	36.3	14.7	0.099	BDL						
	120-135	nd	nd	nd	nd	0.258	0.032						
	135-150	5.2	loam	40.5	9.9	0.118	BDL						
	150-165	nd	nd	nd	nd	0.093	BDL						
	165-180	5.2	loam	44.2	8.1	0.100	BDL						
	180-195	nd	nd	nd	nd	0.056	BDL						
	195-210	5.1	loam	43.2	13.2	0.071	BDL						
	0-30	6.2	loam	47.8	16.7	0.703	0.071	2.1	16.5	51.5	76	571	16
	30-60	6.5	loam	50.7	17.2	0.484	0.048						
	60-90	6.4	loam	29.2	23.8	0.364	0.039						
Howard IB	90-120	6.1	Clay loam	23.7	28.3	0.207	0.034						
	120-150	5.7	loam	30.5	23.9	0.134	0.027						
	150-180	5.9	loam	42.0	19.8	0.139	0.023						
	180-210	5.9	loam	39.9	19.3	0.157	BDL						
	0-15	6.0	loam	38.3	21.9	1.206	0.127	3.1	7.5	62	84.5	1042	5
	15-30	6.6	loam	36.1	26.1	0.397	0.050	2.2	2	42	91.5	998	1.5
	30-45	nd	nd	nd	nd	0.260	0.040						
	45-60	6.5	Clay loam	30.8	27.2	0.295	0.044						
	60-75	nd	nd	nd	nd	0.261	0.037						
Howard IC	75-90	6.0	loam	41.9	22.5	0.113	BDL						
Howard IC	90-105	nd	nd	nd	nd	0.087	BDL						
	105-120	5.8	loam	45.1	18.6	0.092	BDL						
	120-135	nd	nd	nd	nd	0.159	BDL						
	135-150	6.1	loam	44.7	16.1	0.071	BDL						
	150-165	nd	nd	nd	nd	0.087	BDL						
	165-180	5.5	Sandy loam	58.1	13.0	0.066	BDL						

	180-195	nd	nd	nd	nd	0.056	BDL						
	195-210	5.5	Sandy loam	74.7	5.4	0.061	BDL						
	0-15	6.4	loam	50.3	15.0	1.195	0.130	3.5	27	54.5	71	1077	5.5
	15-30	6.5	Sandy loam	59.0	15.8	0.309	0.043	1.95	7	45	70.5	851	4
	30-45	nd	nd	nd	nd	0.153	0.026						
	45-60	6.0	Sandy loam	62.6	15.5	0.125	BDL						
	60-75	nd	nd	nd	nd	0.226	0.033						
	75-90	5.6	Sandy loam	63.1	15.0	0.066	BDL						
Howard ID	90-105	nd	nd	nd	nd	0.070	BDL						
noward ID	105-120	5.7	Sandy loam	58.9	16.5	0.107	BDL						
	120-135	nd	nd	nd	nd	0.367	0.043						
	135-150	5.6	Sandy loam	68.6	11.4	0.045	BDL						
	150-165	nd	nd	nd	nd	0.040	BDL						
	165-180	5.6	Sandy loam	69.7	10.8	0.038	BDL						
	180-195	nd	nd	nd	nd	0.051	BDL						
	195-210	5.5	Sandy loam	71.3	9.8	0.042	BDL						
	0-15	6.7	Silt loam	29.2	20.6	1.952	0.202	4.7	87	154	116	1401	29.5
	15-30	6.3	loam	33.1	22.6	0.953	0.098	3.1	33.5	47.5	84	841	7.5
	30-45	nd	nd	nd	nd	0.286	0.039						
	45-60	6.4	Clay loam	35.3	27.3	0.414	0.049						
	60-75	nd	nd	nd	nd	0.176	0.029						
Lancaster IA	75-90	6.4	loam	44.9	23.3	0.090	BDL						
Lancaster IA	90-105	nd	nd	nd	nd	0.102	BDL						
	105-120	6.3	Sandy loam	52.8	18.2	0.079	BDL						
	120-135	nd	nd	nd	nd	0.266	0.038						
	135-150	5.9	Sandy loam	55.4	17.6	0.095	BDL						
	150-165	nd	nd	nd	nd	0.078	BDL						
	165-180	5.5	Sandy loam	55.5	17.2	0.060	BDL						

	180-195	nd	nd	nd	nd	0.061	BDL						
	195-210	5.4	Sandy loam	62.5	11.0	0.044	BDL						
	0-30	6.1	loam	30.5	25.2	0.869	0.087	3.45	81.5	38	60	763	13.5
	30-60	6.2	Clay loam	38.6	29.9	0.292	0.034						
	60-90	6.1	Sandy clay	51.5	26.5	0.167	BDL						
Lancaster IB	90-120	5.7	Sandy loam	55.1	18.4	0.095	BDL						
	120-150	6.1	Sandy loam	62.3	15.6	0.140	BDL						
	150-180	6.0	Sandy loam	67.4	8.3	0.084	BDL						
	180-210	5.7	Sandy loam	73.7	4.4	0.054	BDL						
	0-15	5.8	Silt loam	26.8	19.1	2.306	0.233	5.95	35	40.5	172	852	17.5
	15-30	6.2	loam	29.3	21.0	0.951	0.106	4.05	14.5	23.5	141	701	5.5
	30-45	nd	nd	nd	nd	0.485	0.067						
	45-60	6.2	Clay loam	31.4	30.5	0.321	0.055						
	60-75	nd	nd	nd	nd	0.262	0.049						
	75-90	5.9	Clay loam	34.2	34.4	0.191	0.042						
York I	90-105	nd	nd	nd	nd	0.121	0.035						
I OIK I	105-120	5.6	Sandy clay	47.6	28.1	0.122	0.036						
	120-135	nd	nd	nd	nd	0.498	0.066						
	135-150	5.3	Sandy clay	50.2	24.9	0.161	0.036						
	150-165	nd	nd	nd	nd	0.185	0.036						
	165-180	5.5	Sandy loam	57.5	18.6	0.175	BDL						
	180-195	nd	nd	nd	nd	0.130	0.032						
	195-210	5.3	Sandy loam	58.9	19.2	0.165	0.029						
	0-15	6.7	loam	36.5	20.8	1.506	0.204	3.65	59	114	51	1368	13
	15-30	6.5	loam	40.5	20.3	0.500	0.120	2.15	10.5	64	55	873	12
Carroll I	30-45	nd	nd	nd	nd	0.232	0.090						
	45-60	6.4	loam	48.6	17.0	0.198	0.087						
	60-75	nd	nd	nd	nd	0.153	0.079						

	75-90	6.4	loam	49.9	13.6	0.093	0.073						
	90-105	nd	nd	nd	nd	0.097	0.075						
	105-120	6.0	Sandy loam	52.9	14.1	0.091	0.075						
	120-135	nd	nd	nd	nd	0.116	0.077						
	135-150	6.0	Sandy loam	54.1	8.5	0.069	0.069						
	150-165	nd	nd	nd	nd	0.058	0.072						
	165-180	5.5	Sandy loam	58.5	6.2	0.060	0.070						
	180-195	nd	nd	nd	nd	0.058	0.075						
	195-210	5.4	Sandy loam	65.9	4.1	0.050	0.073						
	0-30	6.8	Silt loam	17.9	22.0	1.258	0.120	2.9	49	80.5	111	1421	3.5
	30-60	6.7	Silty clay	11.4	33.2	0.588	0.064						
	60-90	6.6	Silty clay	18.4	30.9	0.441	0.049						
Franklin IIB	90-120	5.9	clay	30.6	47.8	0.303	0.044						
	120-150	5.6	clay	27.9	59.9	0.179	0.048						
	150-180	5.1	clay	17.6	54.0	0.141	0.043						
	180-210	5.0	clay	13.9	56.8	0.152	0.045						
	0-15	6.3	loam	28.2	22.2	0.842	0.101	2.55	22.5	203	71	1021	11
	15-30	6.4	Clay loam	30.2	28.2	0.341	0.063	1.8	2.5	47.5	61	1023	5.5
	30-45	nd	nd	nd	nd	0.251	0.057						
	45-60	6.1	Clay loam	35.8	28.1	0.161	0.048						
	60-75	nd	nd	nd	nd	0.213	0.055						
Frederick II	75-90	5.8	Clay loam	36.5	37.5	0.123	0.050						
Tredefick II	90-105	nd	nd	nd	nd	0.125	0.056						
	105-120	5.3	clay	29.6	42.6	0.139	0.056						
	120-135	nd	nd	nd	nd	0.239	0.064						
	135-150	5.2	Clay loam	29.6	38.0	0.143	0.054						
	150-165	nd	nd	nd	nd	0.129	0.053						
	165-180	5.7	Clay loam	41.0	32.0	0.321	0.050						

	180-195	nd	nd	nd	nd	0.118	0.052						
	195-210	5.2	Clay loam	39.0	27.6	0.115	0.050						
	0-30	7.3	loam	29.1	22.5	1.025	0.105	2.2	69.5	73.5	108	1470	3.5
	30-60	7.4	Silty clay	18.7	40.0	0.285	0.041						
	60-90	7.4	clay	18.5	48.5	0.223	0.043						
Frederick IV	90-120	6.6	clay	21.6	46.2	0.163	0.038						
	120-150	6.7	Clay loam	28.3	38.7	0.137	0.033						
	150-180	7.1	clay	26.5	45.9	0.169	0.044						
	180-210	7.7	Clay loam	28.5	36.7	1.557 ⁵	0.039						
	0-15	6.5	loam	43.9	12.6	2.193	0.214	4.2	60.5	126	111	1705	17.5
	15-30	6.5	loam	47.7	15.7	1.126	0.103	2.6	36.5	59.5	104	1464	16.5
	30-45	nd	nd	nd	nd	1.297	0.108						
	45-60	6.8	Clay loam	25.5	32.6	1.423	0.111						
	60-75	Nd	nd	nd	nd	1.324	0.097						
	75-90	6.9	Clay loam	35.3	27.6	0.883	0.062						
Howard IA	90-105	nd	nd	nd	nd	0.682	0.047						
noward IA	105-120	6.6	Silt loam	35.9	10.2	0.724	0.050						
	120-135	nd	nd	nd	nd	0.758	0.058						
	135-150	6.8	Sandy clay	47.7	24.9	0.438	0.030						
	150-165	nd	nd	nd	nd	0.374	0.028						
	165-180	6.7	loam	48.3	18.6	0.363	0.023						
	180-195	nd	nd	nd	nd	0.229	BDL						
	195-210	7.0	Sandy loam	68.9	12.3	0.256	BDL						
	0-30	7.1	Silt loam	10.7	17.1	1.581	0.153	2.75	120	134	196	1626	12.5
	30-60	6.8	Silt loam	14.0	24.4	0.560	0.055						
Lancaster V	60-90	7.0	Silt loam	13.9	23.3	0.352	0.034						
	90-120	7.1	Silt loam	17.2	23.6	0.223	0.025						
	120-150	7.0	loam	30.9	22.0	0.186	BDL						

150-		loam	26.2	26.4	0.132	BDL	 	 	
180-2	-210 7.1	Clay loam	24.7	32.0	0.254	0.030	 	 	

¹ pH by glass combination pH electrode and a pH meter (Metler Toledo InLab®413 combination meter)

² Soil particle size analysis by the Modified Pipette Method. Gavlak, R., D. Horneck and R.O. Miller. 2005. Particle size analysis modified pipette method. Soil, plant and water reference methods for the western region. 3rd ed.

³ Total C and N analysis at University of Maryland Department of Environmental Science and Technology Analytical Lab (LECO CN628 Elemental Analyzer, LECO Corp., St. Joseph, MI) (Nelson and Sommers, 1996); (Matejovic, 1993).

⁴ Soil organic matter (SOM; Loss On Ignition method) and nutrient content by Mehlich3 extraction (P, K, Mg, Ca, Na, S) at WayPoint Analytical, Inc (Richmond, VA)

⁵ Carbon probably originated from inorganic CaCO₃ from limestone parent material

Appendix 2. Detailed procedures for soil coring

Soil cores were taken 0-210 cm deep by hand driving Veihmeyer probes into the ground using a 6.8 kg drop hammer (Veihmeyer, 1929; Dean and Weil, 2009). A 152 cm Veihmeyer probe was used to extract soil from 0-120 cm deep, and a 244 cm or 274 cm Veihmeyer probe was used to extract soil from 120-210 cm deep. After the probe was driven into the ground, a jack and lever system was used to remove the probe from the ground, and the soil was emptied from the probe into a trough (Figure 6).

For 2014, 2015, and 2016, the soil coring and division processes differed slightly. In 2014, the 52 cm probe was driven to 120 cm and emptied, extracting soil from 0-120 cm deep, and the 244 cm probe driven to 210 cm and emptied, extracting soil from 120-210 cm deep. The soil was spread to the appropriate length in the trough—e.g., for the first soil cores it was spread to 120 cm. Soil cores were divided into 15 cm increments. The two soil cores collected from each point along the transect were composited for each depth increment.

In 2015, the 152 cm probe was driven to 60 cm and emptied, extracting soil from 0-60 cm deep, the 152 cm probe was driven to 120 cm and emptied, extracting soil from 60-120 cm deep, and the 244 cm probe was driven to 210 cm and emptied, extracting soil from 120-210 cm deep. The soil was spread to the appropriate length in the trough—e.g., for the first soil core it was spread to 60 cm. Soil cores were divided into 30 cm increments. No soil cores were composited.

In 2016, the 152 cm probe driven to 60 cm and emptied, extracting soil from 0-60 cm deep, the 152 cm) probe driven to 120 cm and emptied, extracting soil from 60-120

cm deep, and the 244 cm) probe driven to 210 cm and emptied, extracting soil from 120-210 cm deep. The soil was spread to the appropriate length in the trough. Soil cores were divided into 15 cm increments. The two soil cores taken from each point along the transect were composited for each depth increment.



Figure 6. Veihmeyer probe, hammer, jack and lever system, and PVC troughs used for taking deep soil cores.

Appendix 3. Soil nitrate and ammonium calculations

Soil NO₃ and NH₄ was measured as mg NO₃-N L⁻¹ or mg NH₄-N L⁻¹. Blank samples (samples of filtered 0.5 *M* K₂SO₄ solution) were included every 20 soil samples. The average concentration of NO₃-N L⁻¹ or NH₄-N L⁻¹ in these blank samples was subtracted from the soil sample NO₃-N L⁻¹ or NH₄-N L⁻¹ for all of the samples analyzed on a particular run of the Lachat instrument, to give the estimated concentration of NO₃-N L⁻¹ and NH₄-N L⁻¹ for the sample (**Equation 2** shows calculation for NO₃-N). The NO₃-N L⁻¹ and NH₄-N L⁻¹ concentrations were then converted to concentrations in soil (**Equation 3** shows calculation for NO₃-N), and the amount of NO₃-N and NH₄-N per area (**Equation 4** shows calculation for NO₃-N). The NO₃-N percent of the total mineral N (NO₃-N + NH₄-N) was determined.

Equation 2 Estimated concentration of NO₃-N in extraction solution

Estimated mg NO_3 -N L^{-1} = Measured mg NO_3 -N L^{-1} in sample – Average of blank mg NO_3 -N L^{-1}

Equation 3 Concentrations of NO₃-N in soil

$$\frac{mg \ NO_3 - N}{kg \ soil} = \frac{mg \ NO_3 - N}{L} * \frac{20 \ ml}{1} * \frac{1 \ L}{1000 ml} * \frac{1}{2} \frac{1}{g \ soil} * \frac{1000 \ g \ soil}{kg \ soil}$$

Equation 4 Amount of NO₃-N per hectare

$$\frac{kg \ NO_3 - N}{ha} = \frac{mg \ NO_3 - N}{kg \ soil} * \frac{1 \ kg \ NO_3 - N}{1000000 \ mg \ NO_3 - N} * \frac{1 \ kg \ soil}{1000 \ g \ soil} * \frac{g \ soil}{cm^3} * \frac{cm \ depth}{1} * \frac{100,000,000 \ cm^2}{1 \ ha}$$

Appendix 4. Soil bulk density values

The number of cores averaged and the depth increments varied by farm (Table 6). The bulk density values for each layer from each farm were analyzed using box and whisker plots in SAS version 9.4 (SAS Institute, Cary, NC). To exclude outliers and possible errors, values beyond 1.5 x Interquartile range (25th to 75th percentiles) were not included when calculating the average bulk density value.

Table 6. Bulk density (BD) mean (g cm⁻³), standard deviation (SD) (g cm⁻³), first (Q1) and third (Q3) interquartile range (25th to 75th percentiles) and number of outliers above fences for all farms in which soil cores were taken. The fence is defined as 1.5 x

Interquartile range (25th to 75th percentiles).

Interquartile range (25	th to 75 th pe	ercentiles).					
Site	Soil depth	# of cores averaged	BD mean (g cm ⁻³)	BD SD (g cm ⁻³)	BD Q1	BD Q3	# of outliers above fences
Caroline I	0-120	5	1.53	0.273	1.27	1.69	1
	120-210	5	1.57	0.0799	1.51	1.65	1
Caroline II	0-120	5	1.36	0.0443	1.33	1.38	1
	120-210	5	1.18	0.102	1.17	1.22	1
Carroll I	0-120	5	1.30	0.135	1.21	1.39	1
	120-210	5	1.01	0.252	0.836	1.03	1
Dorchester IA	0-120	10	1.55	0.178	1.53	1.68	1
	120-210	11	1.22	0.230	1.10	1.40	1
Dorchester IB	0-60	36	1.50	0.125	1.43	1.59	1
	60-120	36	1.72	0.213	1.60	1.87	1
	120-180	35	1.53	0.297	1.37	1.81	1
Franklin I	0-120	5	1.21	0.147	1.15	1.27	1
	120-210	4	1.13	0.385	0.801	1.47	1
Franklin IIB	0-60	11	1.38	0.103	1.29	1.48	1
	60-120	12	1.50	0.171	1.37	1.66	1
	120-210	6	1.65	0.385	1.53	2.01	1
Frederick I	0-120	29	1.63	0.272	1.54	1.66	1
	120-210	25	1.87	0.227	1.71	2.02	1
Frederick II	0-120	5	1.58	0.107	1.50	1.63	1
	120-210	5	1.33	0.197	1.14	1.45	1
Frederick IV	0-60	24	1.39	0.0780	1.33	1.47	1
	60-120	23	1.538	0.138	1.46	1.63	1
	120-210	23	1.38	0.275	1.276	1.62	1
Harford I	0-120	15	1.31	0.273	1.16	1.49	1
	120-210	15	1.19	0.362	1.02	1.44	1
Howard IA	0-120	9	1.43	0.0881	1.35	1.49	1
	120-210	6	0.985	0.281	0.782	1.28	1
Howard IB	0-60	25	1.308	0.0634	1.26	1.34	1
	60-120	25	1.48	0.110	1.38	1.57	1
	120-210	27	1.51	0.280	1.38	1.64	1
Kent I	0-120	4	1.16	0.505	0.824	1.50	1
	120-210	3	1.17	0.330	0.789	1.40	1
Kent II	0-60	24	1.53	0.124	1.44	1.62	1
	60-120	24	1.62	0.0936	1.58	1.66	1
	120-210	23	2.08	0.512	1.56	2.65	1
Lancaster IA	0-120	29	1.22	0.217	1.08	1.43	1
	120-210	28	1.48	0.240	1.35	1.67	1
Lancaster IB	0-60	26	1.14	0.176	1.01	1.23	1
	60-120	25	1.39	0.175	1.28	1.49	1
						•	

	120-210	26	1.44	0.312	1.25	1.57	1
Lancaster V	0-60	40	1.30	0.0814	1.25	1.36	1
	60-120	40	1.63	0.115	1.57	1.70	1
	120-210	38	1.50	0.323	1.35	1.70	1
Prince Georges I	0-120	5	1.36	0.0716	1.30	1.42	1
	120-210	5	1.03	0.184	1.01	1.15	1
St Marys I	0-120	4	1.20	0.0961	1.12	1.28	1
	120-210	4	1.48	0.312	1.28	1.69	1
York I	0-120	4	1.12	0.0721	1.08	1.17	1
	120-210	4	1.00	0.112	0.922	1.08	1
Howard IC, ID	0-60	10	1.29	0.105	1.22	1.33	1
	60-120	10	1.36	0.239	1.17	1.48	1
	120-210	10	1.40	0.136	1.27	1.50	1
Kent IIB, IIC	0-60	10	1.56	0.0970	1.51	1.59	1
	60-120	10	1.48	0.160	1.36	1.55	1
	120-210	10	1.80	0.264	1.63	1.93	1
Kent IID, IIE	0-60	10	1.44	0.126	1.31	1.55	1
	60-120	10	1.38	0.212	1.33	1.45	1
	120-210	10	1.65	0.102	1.54	1.73	1
Prince Georges IIIA, IIIB	0-60	10	1.29	0.160	1.14	1.40	1
	60-120	10	1.37	0.247	1.27	1.45	1
	120-210	9	1.74	0.256	1.56	1.80	1

Chapter 3: Cover crop species and planting date affect deep soil nitrate capture

Abstract

Following summer cash crops, substantial mineral N (100-500 kg N ha⁻¹) remains in the 0-2 m soil, which is at risk to leach during the winter and be out of reach for subsequent crops. We hypothesized that cover crops planted by mid-September could capture residual N, and potentially recycle this N to the following cash crop. We buried ¹⁵N tracer in deep soil layers (60 cm to 200 cm deep) and evaluated the percent recovery of September- and October-planted cover crops of forage radish, rye, and mixtures of forage radish plus rye, with and without crimson clover. In experiment #1, by December, early-planted cover crops recovered on average 13.7% of the buried ¹⁵N from 100 cm deep, while late-planted cover crops recovered only 0.26% from 100 cm deep. In experiment #2, early-planted cover crops recovered on average 14.5% of the buried ¹⁵N from 60 cm deep, while the late-planted cover crops recovered only 1.4%. While the percent recovery of the buried ¹⁵N from 120 cm and 180 cm deep was low in all cases, the early-planted cover crops recovered more ¹⁵N (2.67%) than the late-planted cover crops (0.07%) from 120 cm deep. Early-planted cover crops captured on average 0.31% of the buried ¹⁵N from 180 cm deep. We found limited evidence that late-planted cover crops will capture deep soil residual soil N in the spring, but much smaller amounts than the early-planted cover crops capture in the fall. Early-planted radish and rye species, alone or in mixtures, were capable of quick, deep, root growth and are therefore promising "catch-crops".

Introduction

Leaching as nitrate (NO₃) can be the main pathway for loss of nitrogen (N) from farmland in the Mid-Atlantic USA, especially from November through May when there is little vegetation growing on cropland and precipitation is greater than evapotranspiration (Meisinger, et al., 1991; Shipley, et al., 1992). The uptake of N from the soil by corn (*Zea mays* L.) typically stops by early September (or 100 days after emergence) when corn maturity is approached (Hanway, 1963; Ciampitti, et al., 2013). At this point the NO₃ remaining in the soil and any additional NO₃ that is created by mineralization can begin to leach if water is percolating through the soil. In Pennsylvania, on a Hagerstown silt loam soil, 24-55% of fertilizer N applied at economic optimum rates was leached from the soil (Jemison and Fox, 1994).

Nitrate leaching poses environmental risks, as NO₃ can enter groundwater and bodies of water, such as the Chesapeake Bay. According to the Chesapeake Bay Model, agriculture is responsible for approximately 43% of the N getting into the bay—17% from chemical fertilizer, 19% from manure, and 7% from air deposition of ammonia from livestock (e.g., emissions from poultry houses and dairies) and agricultural soil emissions (Environmental Protenction Agency, 2010). Furthermore, in the Chesapeake Bay watershed, approximately 50% of the N load in streams was transported through groundwater (Phillips and Lindsey, 2013). Excessive N and phosphorus (P) loading in the Chesapeake Bay and its tributaries have caused eutrophication—leading to harmful algal blooms, decreased water clarity, and decreased submerged aquatic vegetation—and periods of hypoxia (dissolved-oxygen concentration < 1.0 mg L⁻¹), stressing and killing aquatic organisms (e.g., shellfish; Ator and Denver, 2015; Phillips and Caughron, 2014).

It is important to note that it is desirable to have plentiful N in the soil.

Agricultural production is dependent on large pools of available N in the soil profile.

However, how deep the N is located is key. Thorup-Kristensen (2006a) noted that the downward leaching of N is not a loss process, but rather the loss occurs if the N leaches beyond the rooting zone of crops. Nitrate that is leaching through the soil profile from August through May will likely be out of reach for the following year's corn crop.

Corn tends to utilize N mostly from soil layers < 50 cm deep, especially in well-fertilized fields (Gass, et al., 1971; Ju, et al., 2007). Corn rooting depth was found to be < 0.8 m at V9 (nine leaf collar) stage and reached a maximum root depth of 1.2 m at silking stage (Zhou, et al., 2008). Some cover crop species have the potential to quickly grow deep roots, and could serve as a "catch crop" to capture NO₃ in the fall months before it leaches out of reach, and potentially release N in the spring months to be used by the following cash crop (Dabney, et al., 2010; Meisinger, et al., 1990; Meisinger, et al., 1991). Deep-rooted cover crops could reach deeper soil layers and capture more N from those layers. For example, Zhou, et al. (2008) found that while corn roots did not reach depths > 1.2 m, subsequent winter wheat (*Triticum aestivum* L.) could use soil NO₃ up to 2 m deep. Huang, et al. (1996) found that corn only removed 1 kg NO₃-N ha⁻¹ from 120 cm deep soil, whereas switchgrass removed 20 kg NO₃-N ha⁻¹.

The ability of cover crops to capture deep soil N has been shown to be dependent upon many factors including cover crop species and planting date and soil characteristics. Three main functional groups of cover crops that are grown include winter cereal grasses, legumes, and brassicas (Dabney, et al., 2010). Research has shown that forage radish (*Raphanus sativus* L. cv. Daikon) and cereal rye (*Secale cereale* L.) are effective at

scavenging deep soil N. Dean and Weil (2009) found that forage radish and cereal rye took up most of the NO₃ from the soil profile, while no-cover crop control plots, particularly in sandy soils, had large pools of NO₃ moving down between 60-90 cm. In the fall, the radish cover crop was more effective than cereal rye or rape (Brassica napus L. cv. Dwarf Essex) at depleting NO₃ from the soil profile and taking up N (Dean and Weil, 2009). Cereal rye is well-documented in its ability to reduce NO₃ leaching. Staver and Brinsfield (1998) found that a rye cover crop following corn reduced annual leaching losses by 80% in comparison to no cover crop. In Kentucky, McCracken, et al. (1994) compared NO₃ leached over the winter (between corn harvest and corn planting) for cereal rye cover crop versus no cover crop in (NH₄)₂SO₄ fertilized zero-tension lysimeters. Compared to the no cover crop treatment, the rye treatment had 0.2% of the loss of NO₃ in year one, 3.6% in year two, and 13.4% in year three (McCracken, et al., 1994). A study using lysimeters in Beltsville, Maryland found that NO₃ leaching was reduced 95% in dry years and 50% in wet years for cover crops of cereal rye, wheat, or barley (*Hordeum vulgare* L.; Meisinger and Ricigliano, 2017).

Root depth and the rate of root growth are important factors determining whether plants are able to acquire N at the times when large amounts of it are available in the soil profile. Measures of root depth, root frequency, and root intensity (root intersections m⁻¹ line on minirhizotron) are all highly correlated with subsoil (0.5-1.0 m) NO₃ uptake (Thorup-Kristensen, 2001; Thorup-Kristensen, 2006a). Forage radish was found to grow roots > 2.4 m deep, and to have root frequencies (percentage of 4 x 4 cm crosses where roots observed on minirhizotron) > 40% down to 2.25 m deep (Thorup-Kristensen, 2006a; Kristensen and Thorup-Kristensen, 2004a). Cereal rye has been found to grow

roots 1.15 m deep (Kristensen and Thorup-Kristensen, 2004a). Forage radish reached 1 m deep with fewer growing degree days than cereal rye (Kristensen and Thorup-Kristensen, 2004a).

Planting cover crops earlier in the fall can make a significant difference in the ability of cover crops to capture N. Planting cover crops earlier allows cover crops to utilize more growing degree days. Lacey and Armstrong (2015) found that colder weather conditions likely contributed to less biomass accumulation and N uptake in forage radish compared to cereal rye. Earlier planting also reduces the depth of rooting required to "catch-up" with NO₃ that is likely to be leaching deeper in the soil profile throughout the fall and winter.

Nitrogen that is captured by cover crops can be a valuable resource for farmers if it is released into the soil as available N in synchrony with cash crop N uptake needs. The release of scavenged N is important for improving the overall N use efficiency of the cropping system. For example, while winter cereals are effective at N scavenging, N in their residues is released very slowly by decomposition and is often immobilized by microbes utilizing the abundant carbon in the residues and is therefore largely unavailable for crop uptake. As a result, higher levels of spring N fertilizer are often applied following winter cereal cover crops than would be applied without a cover crop. Legume cover crops foster microbial N fixation, which adds N to the system. Legume residues also have a relatively low C/N ratio which improves N availability following cover crop decomposition. Legume cover crops result in fertilizer credits (reduced fertilizer rates) ranging from 56-135 kg N ha⁻¹, whereas N credits from grass cover crops are usually negative, requiring that additional fertilizer be applied to the following crop (Doran and

Smith, 1991; Meisinger, et al., 1990). Poffenbarger, et al. (2015a) found that at the end of the corn growing season, cereal rye had released only 8.5 kg N ha⁻¹, while hairy vetch (*Vicia villosa* Roth) had released 280 kg N ha⁻¹ and a 50/50 mix of rye/vetch had released 139 kg ha⁻¹. Kramberger, et al. (2009) found that ryegrass (*Lolium multiflorum* Lam.) and rape (*Brassica napus* ssp. *Oleifera* (Metzg.) Sinsk) cover crops significantly depleted fall and spring soil mineral N in the 0-90 cm soil profile, whereas subclover (*Trifolium subterraneum* L.) and crimson clover (*Trifolium incarnatum* L.) decreased soil mineral N to a lesser extent and less frequently; however, the clovers tended to increase the following corn yield and corn N content, while rape had no effect on corn yield and corn N content.

Because legumes are N-fixing, there is concern that including a legume within a mixed species cover crop will impede the ability for the cover crop to scavenge soil NO₃. For example, prior to 2015, if farmers planted mixed-species cover crops that included a legume, they were not eligible for incentive payments through the Maryland Department of Agriculture cover crop program (Maryland Department of Agriculture, 2015).

There are challenges studying rooting patterns and nutrient uptake by plants deep in the soil. Deep soil coring is time-consuming and laborious. In addition, soils and root systems are more heterogeneous in deeper layers than in topsoil layers. For example, measurements of soil organic carbon (SOC) had a higher coefficient of variation (80.2%) in the subsoil (30-40 cm depth) than in the topsoil (0-10 cm depth) (34.4%) (Usowicz and Lipiec, 2017). In addition, root intensity and root frequency is greatly reduced and therefore more spatially heterogeneous below 1 m deep (Kristensen and Thorup-Kristensen, 2004a). Therefore a greater number of core samples are needed to estimate

parameters with confidence, but a smaller number of cores are usually dictated by logistical considerations. Root studies often underestimate root activity by not accounting for fine roots or root turnover with time (Dabney, et al., 2010). In addition, N uptake by individual species within plant mixtures usually cannot be differentiated (Maeght, et al., 2013). Isotopic tracers can be used to assess nutrient uptake from various depths (Maeght, et al., 2013). Injecting ¹⁵N, a nonradioactive heavy isotope, to a subsurface soil depth is a common method for assessing N uptake by crops or cover crops (Andersen, et al., 2014; Gathumbi, et al., 2003; Ju, et al., 2007; Kristensen and Thorup-Kristensen, 2004a; Kristensen and Thorup-Kristensen, 2004b; Ramirez-Garcia, et al., 2014; Yang, et al., 2014).

In the present study, we buried K¹⁵NO₃ tracer and planted cover crops over the burial points. This allowed us to investigate cover crop uptake from pools of NO₃ that were present at a particular depth in late-August. We chose to bury the ¹⁵N in late-August because corn in the study region stops taking up soil N at this time. The purpose of this study was to investigate whether NO₃ that remains in the subsoil (100 or 200 cm in year one, and 60, 120, or 180 cm in year two) at the end of the corn growing season can be captured by cover crops. Specifically, our objectives were:

- 1. Evaluate the ability of four cover crops—radish, rye, radish + rye in mix, radish + rye + crimson clover in mix—to capture deep soil NO₃;
- 2. Measure the effect of cover crop planting date on the ability of these cover crops to capture deep soil NO₃;
- 3. Determine if summer corn following no cover crop can capture NO₃ that was 60,120, or 180 cm deep the previous August, and investigate if corn following a

cover crop is more likely to contain the NO₃ that was 60 cm deep the previous August than corn following no cover crop.

Materials and methods

Experiment #1

Study sites

The study was located at the Central Maryland Research and Education Center—Beltsville Facility in Laurel, Maryland USA. The region has a humid climate with annual rainfall relatively uniformly distributed throughout the entire year. The average annual precipitation for Beltsville, MD is 1063 mm (US Climate Data, 2018). The temperature and precipitation for the study period is found in Figure 7. The experiment was performed from September 2014 to November 2014 during the fall cover crop growing season. The study contained six blocks, three at each of two sites, located 1.30 km away from each other. Site one (39.010638, -76.832985) had a Russett soil, with a loamy fine sand surface horizon texture. Site two (39.018457, -76.820808) had a Christiana soil, with a clay loam surface horizon. Table 7 lists soil pH, percent sand, percent clay, soil texture, percent C, and percent N of the study site soils for each 20 cm depth increment from 0-200 cm, and P, K, Mg, Ca, and S (mg kg⁻¹) for 0-40 cm. Prior to the study, the sites both had wheat with a double crop of soybean in 2014, soybean in 2013, and corn in 2012.

Experimental design and treatments

The study included 12 treatments in a split-split plot design with three replications at both sites. Plots were 3 m x 3 m in size. Experimental factors that defined the

treatments included cover crop planting date, cover crop species, and ¹⁵N burial treatments. The treatments were in a complete factorial combination of these factors with cover crop planting date as the main plot factor, cover crop species as the split-plot factor, and ¹⁵N burial depth as the split-split plot factor (Figure 8). The cover crop treatment included: 1) forage radish (radish) and 2) cereal rye (rye). Rye is a common cover crop grown in Maryland and the cover crop with the largest monetary incentives under the Maryland cover crop program (Maryland Department of Agriculture, 2018). Radish is a cover crop increasingly being used in Maryland, which has shown much potential for quick root growth and deep N scavenging. The cover crop planting date included: 1) an early-planted date of 28 Aug and 2) a late-planted date of 29 Sep. The ¹⁵N burial depth treatments included: 1) 100 cm burial, 2) 200 cm burial, and 3) control treatment in which no ¹⁵N was applied.

Field operations

Double-crop soybeans at reproductive one (R1) stage were mowed (soybean residue remained on field) on 26 Aug 2014 to accommodate the planting date of the study. On 26 Aug 2014, the early-planting date plots were sprayed with Paraquat at the rate of 0.841 kg active ingredient ha⁻¹ for weed control and ammonium sulfate fertilizer at a rate of 22.4 kg N ha⁻¹. Early-planted cover crops were planted 28 Aug 2014. On 27 Sep 2014, the late-planting date plots were sprayed with Paraquat at the rate of 0.841 kg active ingredient ha⁻¹ for weed control and ammonium sulfate fertilizer at a rate of 22.4 kg N ha⁻¹. Late-planted cover crops were planted 29 Sep 2014. All cover crops were planted using a Great Plains Solid Stand 10, no-till drill.

¹⁵N solution and burial

Solution of 0.5 g KNO₃ isotopic tracer 99% enriched in 15 N and 250 ml DI (deionized) water were made. Each solution contained 0.07345 g 15 N (Equation 5). The 15 N solution was buried at one point in the center of each plot.

Equation 5 Amount of ¹⁵N tracer buried per hole

$$0.5 \text{ g K}^{15} \text{NO}_3 * \frac{15.00 \text{ g}^{15} \text{N}}{102.10 \text{ g K}^{15} \text{NO}_3} = 0.07345 \text{ g}^{15} \text{N}$$

After cover crops emerged, the burial point was selected to be in a good stand of cover crop, ideally near center of the 3 m x 3 m plot. A bore hole 7.0 cm in diameter were made vertically to the desired soil depth using a bucket auger, and a 5.1 cm PVC pipe was immediately inserted into the hole. The 250 mL of the K¹⁵NO₃ solution was poured in the hole, followed by 50 ml of DI (deionized) water to rinse the pipe. Each hole was filled with the removed subsoil to approximately 15 cm above the K¹⁵NO₃, followed by a 2:1 sand/bentonite mix until the hole was filled up to within 30 cm from the surface. The bentonite mix was used to prevent preferential root growth down the backfilled hole. The top 30 cm of the hole was filled with topsoil from the plot.

Cover crop biomass sampling at ¹⁵N burial points and preparation for analysis

Cover crop biomass was harvested 25 Nov 2014. Weeds were not harvested because they were estimated to be < 5% of biomass. The biomass was harvested if the point where it emerged from the soil fell within a 30 cm radius of the burial point. The radish fleshy taproot (radish root) was pulled from the ground with the leafy top (radish shoot) attached. The root and the shoot were broken apart, and the root was washed. Rye was cut 1 cm above the soil surface. The radish root, radish shoot, and rye tissue types were analyzed separately, as different plant parts may be expected to accumulate

different amounts of ^{15}N (Quemada and Cabrera, 1995). Cover crop biomass samples were dried at 40° C, weighed, and ground to < 0.1 mm size. The dry matter per m^2 for each tissue type and for each cover crop (sum of tissue types; e.g., radish = radish shoot + radish root) was calculated.

Cover crop biomass

By 25 Nov 2014, early-planted rye accumulated on average 2500 kg ha⁻¹ dry matter (SE = 160), and early-planted radish shoot accumulated 2600 kg ha⁻¹ dry matter (SE = 180) and root accumulated 1700 kg ha⁻¹ dry matter (SE = 70). Late-planted rye accumulated on average 780 kg ha⁻¹ dry matter (SE = 70), and late-planted radish shoot accumulated 910 kg ha⁻¹ dry matter (SE = 90) and root accumulated 210 kg ha⁻¹ dry matter (SE = 20).

Soil sampling at ¹⁵N burial points and preparation for analysis

In order to investigate NO_3 leaching patterns, soil cores were taken 10 cm to the side of the buried ^{15}N tracer on 7 Dec 2014 and 18 May 2015. In December, at site one, a soil core was taken in two of the early-planted rye plots in which the ^{15}N tracer was buried 100 cm deep. Soil cores were taken to 165 cm deep, and the 90-165 cm soil was divided into 15 cm increments. In May, at sites one and two, soil cores were taken in three of the early-planted rye plots in which the ^{15}N tracer was buried 100 cm deep. Soil samples were dried at $40^{\circ}C$ for at least 48 hours. The soil was sieved through a 2 mm sieve and then ground to < 0.1 mm. Soil samples from 90-165 cm depths were analyzed for ^{15}N .

¹⁵N analysis and calculations

Biomass and soil samples were analyzed for ¹⁵N at Cornell University Stable

Isotope Laboratory using an isotope ratio mass spectrometer (ThermoFinnigan Delta

Plus) integrated with an elemental analyzer (Carlo Erba NC2500) through an open split

interface (Conflo II). The concentration of ¹⁵N in the sample is reported as atom percent

(at%) ¹⁵N. The amount of ¹⁵N uptake per m² was calculated using **Equation 6** and **4**. The

percent of ¹⁵N that was recovered from the total amount buried was calculated using **Equation 8** and **6**.

Equation 6. Tissue type ¹⁵N Uptake

$$\frac{g^{15}N \text{ uptake of tissue type}}{m^2} = \frac{g \text{ dry weight}}{m^2} * \frac{\% \text{ N}}{100} * \frac{\text{at}\%^{15}N \text{ excess}_{\text{sample}}}{\text{at}\%^{15}N \text{ excess}_{\text{fertilizer}}}$$

Where,

at%
15
N excess_{sample} = at% 15 N_{sample} - at% 15 N_{control} at% 15 N excess_{fertilizer} = at% 15 N_{fertilizer} - at% 15 N_{control} at% 15 N_{fertilizer} = 99.0 at% 15 N_{control} = average of the 18 control no 15 N samples with the same cover crop planting date as the sample

Equation 7. Cover crop ¹⁵N uptake

$$\frac{g^{15}N \text{ uptake}_{\text{Cover crop}}}{m^2} = \sum \frac{g^{15}N \text{ uptake}_{\text{Tissue type}}}{m^2}$$

Where,

Radish had two tissue types: 1) radish shoot, 2) radish root

Rye had one tissue type: 1) rye

Equation 8. Tissue type percent ¹⁵N recovery

15
N percent recovery_{Tissue type} = $\left(\frac{g^{15}N \text{ uptake}}{\text{plot area}} / \frac{g^{15}N \text{ buried}}{\text{plot area}}\right) * 100$

Where,

$$g^{15}N$$
 buried = 0.07345 g

Plot area =
$$\pi * (0.3 \text{ m})^2 = 0.28 \text{ m}^2$$

Equation 9. Cover crop ¹⁵N percent recovery

 15 N percent recovery $_{Cover\ crop} = \sum{}^{15}$ N percent recovery $_{Tissue\ type}$

Where,

Radish had two tissue types: 1) radish shoot, 2) radish root

Rye had one tissue type: 1) rye

Statistical analysis

All analyses were performed using SAS version 9.4 statistical software (SAS Institute, Cary, NC). For all tests, the level of probability considered significant was p < 0.05, unless otherwise stated. We evaluated if cover crops from experimental treatments contained higher at% 15 N than background level. The 15 N isotope occurs naturally in an almost constant ratio of 1:272 for at% 15 N/ 14 N. In other words, 0.366% of N has a mass of 15 rather than 14 (Hauck, et al., 1994). This natural background at% 15 N can be compared to the sample at% 15 N in order to determine if the sample is enriched or not (Hauck, et al., 1994). The background levels (unenriched) of at% 15 N of cover crop tissue were determined by measuring the at% 15 N for each cover crop tissue type in control (no 15 N) plots. An analysis of variance (ANOVA) was performed to test if the background at% 15 N varied due to fixed effects of 1) cover crop tissue type, 2) cover crop planting date, 3) site, 4-7) all interactions of these factors. The site one, rep 3, late-planted, radish

root at% 15 N value (0.40082) was excluded from the analysis due to being > 5 standard deviations greater than the mean.

The ANOVA showed a significant effect (p = 0.0047) for cover crop planting date on the background levels of at% 15 N, with early-planting (at% 15 N = 0.3699) higher than late-planting (at% $^{15}N = 0.3683$). Because there were no differences between sites or among tissue types, the background at% ¹⁵N values were pooled over these variables. The at% ¹⁵N of the cover crop tissue in an enriched plot was compared to the at% ¹⁵N of the cover crop tissue in an unenriched plot (background level) with the same cover crop planting date. A one-sample t-test, which compared a given value to a sample mean, was performed to determine if an experimental treatment value of at% ¹⁵N was significantly higher than the background level. Because we were only interested in knowing if cover crops from experimental treatments had a higher at% ¹⁵N than the background level (not lower), a "lower one-sided t-test" was performed. The null value (H0) was the at% 15N of the experimental plot in question. The null hypothesis is that the mean of background at% ¹⁵N values is equal to the H0; the alternative hypothesis is that the mean of background at% ¹⁵N values is less than the H0. Levels of at% ¹⁵N significantly above the background at% ¹⁵N values were interpreted to mean that the cover crop captured some of the buried ¹⁵N tracer.

We evaluated how much ¹⁵N buried tracer the cover crops captured. An ANOVA was performed to determine if ¹⁵N percent recovery was affected by the fixed effects of 1) cover crop, 2) cover crop planting date, 3) ¹⁵N burial depth, 4) species x planting date, 5) species x ¹⁵N burial depth, 6) planting date x ¹⁵N burial depth, 7) species x planting date x ¹⁵N burial depth, and 8-14) the interactions of all variables above x site. Random

effects were rep(site) and rep x planting date, and rep x species x planting date. The ¹⁵N percent recovery response data was not normally distributed so it was log10 transformed, which normalized the distribution. Thus the dependent variable in the ANOVA was log10 transformed ¹⁵N percent recovery.

Experiment #2

Study sites

The study was located at the Central Maryland Research and Education Center—Beltsville Facility in Laurel, Maryland USA. The region has a humid climate with annual rainfall relatively uniformly distributed throughout the entire year. The temperature and precipitation for the study period is found in Figure 9. The experiment was performed from September 2015 to October 2016 during the cover crop-cash crop cycle. The study contained six blocks, three at each of two sites, located 1.06 km away from each other. Site three (39.01162, -76.83167) and site four (39.01837°, -76.82247°) soils were primarily Russett, with a sandy loam surface horizon. Table 8 lists soil pH, percent sand, percent clay, soil texture, percent C, and percent N of the study site soils for each 30 cm depth increment from 0-210 cm, and P, K, Mg, Ca, and S (mg kg⁻¹) for 0-30 cm. Prior to the study, both fields were planted in winter wheat fall 2014-summer 2015. The fields were in corn in 2014, and in fall 2012-summer 2013 the fields were in winter wheat with a double crop of soybean.

Experimental design and treatments

The study included 29 treatments in a randomized complete block design with six replications. Plots were 3 m x 3 m in size. Experimental factors that defined the treatments included cover crop, cover crop planting date, and ¹⁵N burial depth. The

treatments were in an incomplete factorial combination of these factors. The cover crop treatment included: 1) radish, 2) rye, 3) radish + rye (two-way mix), 4) radish + rye + crimson clover (three-way mix), and 5) control treatment in which no cover crop was planted. In experiment #2, we added the multi-species cover crop mixture treatments that were not included in experiment #1 in order to investigate how radish and rye perform together versus in monoculture, and to asses if the ¹⁵N percent recovery of the rye + radish mix would change with the presence of a legume in the mix.

The cover crop planting date treatments included: 1) an early-planting date of 3 Sep and 2) a late-planting date of 8 Oct. There was no late-planting date treatment for the two-way mix, as we did not expect an interaction between planting date and the influence of clover on the cover crop mixture. The ¹⁵N burial depth treatments included: 1) 60 cm burial, 2) 120 cm burial, 3) 180 cm burial, and 4) control treatment in which no ¹⁵N was applied. There was no 180 cm burial for the late-planting date cover crops, as we did not anticipate cover crops from late-planting reaching even the 120 cm depth (Table 9).

Field operations

Winter wheat was harvested from the plots mid-July 2015, and weeds were killed on 1 Sep 2015 using Glyphosate, N-(phosphonomethyl)glycine at the rate of 2.31 kg active ingredient ha⁻¹. On 3 Sep 2015, the early-planting date plots were fertilized with 9.07 kg N as urea and ammonium nitrate (UAN), and cover crops were planted. On 28 Sep 2015, the late-planting date plots were fertilized with 9.07 kg N (as UAN). Late-planted cover crops were planted 8 Oct 2015. Cover crops were chemically terminated using Glyphosate, N-(phosphonomethyl) glycine at the rate of 2.31 kg active ingredient ha⁻¹ on 18 May 2016.

At site three, corn was planted 16 May 2016. Fertilizer was applied 16 May at planting (45 kg N ha⁻¹) and 14 June 2016 (80.7 N ha⁻¹ and 18.3 kg S ha⁻¹). At site four, corn was planted 7 June 2016. On 8 June 2016, herbicides were applied (1.85 kg ha⁻¹ 2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine (Atrazine), 3.36 kg ha⁻¹ 1,1'-dimethyl-4,4'-bipyridinium dichloride (Paraquat), 1.68 kg ha⁻¹ Acetochlor (Warrant), 0.56 kg ha⁻¹ alcohol ethoxylate, alkylphenol ethoxylate (De-Fac 820 adjuvant non-ionic surfactant). Fertilizer was applied 7 June at planting (45 kg N ha⁻¹) and 29 June 2016 (80.7 N ha⁻¹ and 18.3 kg S ha⁻¹).

¹⁵N solution and burial

Solutions of 0.5 g KNO₃ isotopic tracer 99% enriched in ¹⁵N and 250 ml DI (deionized) water were made. Each solution contained 0.07345 g ¹⁵N (Equation 5). The ¹⁵N tracer was divided among five points per plot. Bore holes 2 cm in diameter were made vertically in the soil using a Veihmeyer probe. Five holes were spatially arranged in the shape of an x, with one hole at the intersection and one at the end of each arm with a distance of 43 cm between the center point and the end of each arm (Figure 10). Immediately following the creation of the five holes in a plot, a PVC pipe was inserted into each hole. Using a funnel, 50 mL of the K¹⁵NO₃ solution was poured in each of the five holes, followed by 10 ml of DI water to rinse the pipe. A total of 250 mL K¹⁵NO₃ solution was buried per plot. Each hole was filled with clean quartz sand to approximately 15 cm above the K¹⁵NO₃, followed by a 2:1 sand/bentonite mix until the hole was filled up to within 30 cm from the surface. The bentonite mix was used to prevent preferential root growth down the backfilled hole. The top 30 cm of the hole was filled with topsoil from the plot.

Cover crop biomass sampling at ¹⁵N burial points and preparation for analysis

We sampled the cover crops with the intention to allow the cover crops to follow their natural process of growth and decomposition as closely as possible. Fall cover crop biomass was sampled between 14 Dec 2015 and 31 Dec 2015. Spring biomass was sampled between 30 April 2016 and 7 May 2016. A plant was sampled if the point where it emerged from the soil fell within a 20 cm radius of each of the five burial points (Figure 10). The tissue types of radish shoot, radish root, rye, and clover shoot were sampled within all treatments. The sampling scheme accounted for the expected differences in C/N ratio and N concentration between the rye and clover stems and leaves (Quemada and Cabrera, 1995). Weeds were not collected because they were estimated to be < 5% of biomass.

Radish was expected to naturally winter-kill within one month of the fall sampling date. Therefore we destructively sampled the radish, but returned > 95% of the biomass to the plot to decompose. Radish was harvested by hand-pulling the fleshy root from the soil. The radish root and shoot were separated. The root was thoroughly washed to remove all soil and then weighed. A 4.40 mm diameter bore was taken horizontally through the root (perpendicular) at the vertical midpoint of the root. The root (minus the bore hole) was returned to the exact point in the soil from which it came. The radish shoot was weighed, and split into two equal parts down the midline of the plant. One half of the radish shoot was scattered over the replaced radish root, and the other half of the radish shoot was blended with 30 ml of DI water until liquefied using a food processor. A sample of 30 ml of the blended radish shoot was saved. The remaining radish shoot puree was immediately stored in the refrigerator and scattered within 48 hours over the burial

points from the plot harvested. The radish biomass was estimated using percent moisture estimates from a previous study (Equation 10).

Equation 10. Estimating radish shoot and root dry matter

$$\frac{g \text{ dry matter}}{m^2} = \frac{g \text{ wet biomass}}{m^2} * \frac{(100 - \% \text{ moisture})}{100}$$

Where,

Wet biomass = values taken in field measurements

Percent moisture = values estimated based on a 2012 radish variety trial; average radish shoot percent moisture = 97.07% (N = 20; SD = 1.09), average radish root percent moisture = 93.55% (N = 20; SD = 1.89) (Lounsbury and Weil, unpublished)

Rye and clover are expected to overwinter in the study region. Therefore we took minimally-destructive samples of the biomass of these species in the fall and in the spring. For rye, one shoot at the base of the stem was sampled from each clump. A "clump" was defined as all rye leaves coming from a single shoot off a tiller. For clover, one shoot was collected at its base from each clump. A "clump" was defined as all clover stems coming from what appeared to be a single root. To reduce human error, a single investigator identified "clumps" for all plots within a replication.

In order to estimate rye biomass, we took measurements of rye patchiness, height, and percent cover. Patchiness was determined by counting the number of clumps. Height was estimated to be the average height of rye leaves within each clump. The leaves were pulled vertical beside a measuring stick to determine the average height. To reduce human error, a single investigator determined the patchiness and height of all plots within a block. To estimate percent cover, a bird's eye photo was taken of each sampling point;

any radish in the plot was removed before the photo was taken. Two investigators made independent visual estimates of percent cover from the photo images; the two estimates were averaged for each point. On areas outside of the plots, we measured rye patchiness, height, and percent cover and then harvested the area and dried and weighed the biomass in order to correlate measurements to actual biomass. We ran a regression analysis to correlate the measured rye parameters to the dry biomass. The analysis selected for the combination of independent variables with the highest adjusted R². We forced the equation to pass through the origin (i.e., have no y intercept) (Eisenhauer, 2003). Correlation curves were made for each sampling group (Appendix 5, Table 15). The dry matter per m² for each tissue type and for each cover crop (sum of tissue types; e.g., radish = radish shoot + radish root) was estimated. Crimson clover biomass was not estimated as we did not expect Crimson clover to reach and take up ¹⁵N tracer.

The average % N for the minimally-destructive and harvested areas outside of the study plots was compared. For the fall samples (N=20), the ratio of % N from sample to total harvest was on average 0.991, with each sample differing on average 7.86% from the total harvest. For the spring samples (N=40), the ratio of % N from sample to total harvest was on average 1.01, with each sample differing on average 10.4% from the total harvest.

Cover crop biomass

December dry matter accumulation for early-planted cover crops was estimated to be $4300 \text{ kg ha}^{-1} \text{ (SE} = 270)$ for two-way mix (radish shoot and root, rye), $4000 \text{ kg ha}^{-1} \text{ (SE} = 230)$ for three-way mix (radish shoot and root, rye, excluding clover), $3900 \text{ kg ha}^{-1} \text{ (SE} = 330)$ for radish (shoot and root), and $1900 \text{ kg ha}^{-1} \text{ (SE} = 90)$ for rye. December dry

matter accumulation for late-planted cover crops was estimated to be 400 kg ha^{-1} (SE = 40) for three-way mix (radish shoot and root, rye, excluding clover), 460 kg ha^{-1} (SE = 50) for radish (shoot and root), and 990 kg ha^{-1} (SE = 70) for rye.

Corn sampling at ¹⁵N burial points and preparation for analysis

Corn plants were sampled when plants had reached the five leaf collar growth stage (V5), on 8 Jun 2016 (for site three) and on 29 Jun 2016 (for site four). Corn grain was sampled on 2 Sep 2016 (for site three) and on 7 Oct 2016 (for site four). Corn was sampled from a 1.8012 m² circular area (0.7572 cm radius) encompassing the five sampling points. The sampling area was split in half; the corn from one half was harvested at V5 stage and the corn from the other half was harvested for grain. The corn biomass was dried at 40° C and weighed. The dried biomass was ground to < 0.1 mm size.

Soil sampling at ¹⁵N burial points and preparation for analysis

In order to investigate NO_3 leaching patterns, soil cores were taken at either 10 cm or 20 cm distance to the side of the buried ^{15}N at several times during the year in the plots that had no cover crops (Table 10). The first set of samples was taken at site three on 20 February 2016 and at site four on 10 April 2016. We intended to take these soil cores in late-fall, but were delayed until February and April by weather and field soil conditions. Soil cores were taken to 210 cm deep and divided into 15 cm increments. Soil samples were dried at $40^{\circ}C$ for at least 48 hours. The soil was sieved through a 2 mm sieve and then ground to < 0.1 mm size. We analyzed soil samples from selected depths for at% ^{15}N (Table 10).

¹⁵N analysis and calculations

Biomass and soil samples were analyzed for ¹⁵N at Cornell University Stable
Isotope Laboratory using an isotope ratio mass spectrometer (ThermoFinnigan Delta
Plus) integrated with an elemental analyzer (Carlo Erba NC2500) through an open split
interface (Conflo II). The ¹⁵N is reported as atom at% ¹⁵N. The ¹⁵N uptake was calculated
using **Equation 11** and **9**, and the ¹⁵N percent recovery was calculated using **Equation**13 and 11.

Equation 11. Tissue type ¹⁵N uptake

$$\frac{\text{g }^{15}\text{N uptake of Tissue type}}{\text{m}^2} = \frac{\text{g dry weight}}{\text{m}^2} * \frac{\% \text{ N}}{100} * \frac{\text{at}\% \ ^{15}\text{N excess}_{\text{sample}}}{\text{at}\% \ ^{15}\text{N excess}_{\text{fertilizer}}}$$

Where,

at%
$$^{15} N \; excess_{sample} = \; at\% \; ^{15} N_{sample} - \; at\% \; ^{15} N_{control}$$

at%
15
N excess_{fertilizer} = at% 15 N_{fertilizer} - at% 15 N_{control}

at%
$$^{15}N_{\text{fertilizer}} = 99.0$$

at% $^{15}N_{control}$ = average of the three control no ^{15}N samples with the same tissue type and site as the sample

Equation 12. Cover crop ¹⁵N uptake

$$\frac{g^{15}N \; uptake_{\text{Cover crop}}}{m^2} = \sum \frac{g^{15}N \; uptake_{\text{Tissue type}}}{m^2}$$

Where,

Radish had two tissue types: 1) radish shoot, 2) radish root

Rye had one tissue type: 1) rye shoot

Two-way mix had three tissue types: 1) radish shoot, 2) radish root, 3) rye shoot

Three-way mix had four tissue types: 1) radish shoot, 2) radish root, 3) rye shoot, 4) clover shoot

Equation 13. Tissue type ¹⁵N percent recovery

15
N percent recovery_{Tissue type} = $\frac{g^{15}$ N uptake / $\frac{g^{15}$ N buried plot area

Where,

for cover crop species,

$$g^{15}N$$
 buried = 0.07345 g

Plot area =
$$5*(\pi (0.2 \text{ m})^2 = 0.62832 \text{ m}^2$$

for corn V5 plants or grain,

g ¹⁵N buried = 0.25 g K¹⁵NO₃ *
$$\frac{15.00 \text{ g}^{15}\text{N}}{102.10 \text{ g} \text{ K}^{15}\text{NO}_3}$$
 = 0.03673 g

Plot area =
$$0.5*(\pi (0.7572 \text{ m})^2 = 0.9006 \text{ m}^2$$

Equation 14. Cover crop ¹⁵N percent recovery

 ^{15}N percent recovery}{\text{Cover crop}} = \sum~^{15}\text{N} percent recovery}{\text{Tissue type}}

Where,

Radish had two tissue types: 1) radish shoot, 2) radish root

Rye had one tissue type: 1) rye shoot

Two-way mix had three tissue types: 1) radish shoot, 2) radish root, 3) rye shoot

Three-way mix had four tissue types: 1) radish shoot, 2) radish root, 3) rye shoot, 4)

clover shoot

Statistical analysis

All analyses were performed using SAS version 9.4 statistical software (SAS Institute, Cary, NC). For all tests, the level of probability considered significant was p < 0.05, unless otherwise stated. We evaluated if cover crops from experimental treatments contain higher at% ¹⁵N than background level. The background levels (unenriched) of at% ¹⁵N of cover crop tissue were determined by measuring the at% ¹⁵N for each cover crop tissue type in control (no ¹⁵N) plots. An ANOVA was performed to test if the background at% ¹⁵N varied due to fixed effects of 1) cover crop tissue type, 2) site, and 3) tissue type x site. Due to the incomplete factorial design, data from early-planted plots was analyzed separately from data from late-planted plots. Fall and spring biomass samples were analyzed separately. For the fall, early-planted cover crops, there were significant effects for site (p = 0.0179) and tissue type (p = 0.0008) on the background levels of at% 15 N. Site four (at% 15 N = 0.3693) was significantly higher than site three (at% $^{15}N = 0.3681$). Clover (at% $^{15}N = 0.3669$) was significantly lower than radish shoot (at% $^{15}N = 0.3700$) and radish root (at% $^{15}N = 0.3696$). Rye (at% $^{15}N = 0.3682$) was significantly lower than radish shoot. For the fall, late-planted cover crops, there were significant effects for site (p = 0.0010) and tissue type (p = 0.0407). Site four (at% 15 N = 0.3699) was significantly higher than site three (at% $^{15}N = 0.3678$). Clover (at% $^{15}N =$ 0.3675) was significantly lower than radish shoot (at% $^{15}N = 0.3701$). For the spring, early-planted cover crops, there was a significant effect for tissue type (at% $^{15}N =$ 0.0236), but not site. Clover (at% $^{15}N = 0.3666$) was significantly lower than rye (at% $^{15}N = 0.3686$). For the spring, late-planted cover crops, there was no significant effect for tissue type or site. Because there were differences between sites and/or among tissue types for the early-planted fall samples, late-planted fall samples, and early-planted

spring samples, the background at% ¹⁵N values for each site x tissue type was analyzed separately. For each site x tissue type, an ANOVA was run to test for differences in the background at% ¹⁵N values among tissue types within different cover crops (e.g., radish shoot in radish cover crop versus radish shoot in three-way mix cover crop). For fall and spring, for each site x tissue type, there were no significant effects of tissue types within different cover crops, and therefore the background level at% ¹⁵N values were pooled over this factor.

The at% ¹⁵N of a tissue type in an enriched plot was compared to the at% ¹⁵N of the same site, tissue type, and planting date in an unenriched plot (background level). A one-sample t-test was performed to determine if an experimental treatment value of at% ¹⁵N was significantly higher than the background level. Because we were only interested in knowing if cover crops from experimental treatments had a higher at% ¹⁵N than the background level (not lower), we used a "lower one-sided t-test", with the null value (H0) being the at% ¹⁵N of the experimental plot in question. The null hypothesis is that the mean of control values is equal to the H0; the alternative hypothesis is that the mean of control values is less than the H0. Levels of at% ¹⁵N significantly above the background level (control) at% ¹⁵N were interpreted to mean that the cover crop captured some of the buried ¹⁵N tracer.

We evaluated how much ¹⁵N buried tracer the cover crops captured. Due to the incomplete factorial design (no two-way mix cover crop planted late, and no ¹⁵N buried at 180 cm for the late-planted cover crops), the results from the early-planted cover crops and late-planted cover crops were analyzed separately. An ANOVA was performed to determine if the ¹⁵N percent recovery was affected by the fixed effects of 1) cover crop,

2) ¹⁵N burial depth, 3) species x ¹⁵N burial depth, and 4-6) the interactions of all variables above x site. The ¹⁵N percent recovery data was not normally distributed so it was log10 transformed, which normalized the distribution. Thus, the dependent variable in the ANOVA was log10 ¹⁵N percent recovery.

To test for differences of ¹⁵N percent recovery between cover crop planting dates, an ANOVA was performed including the independent variables of cover crop, cover crop planting date, ¹⁵N burial depth, all possible interactions, and the interaction of site with all of the above factors. Experimental units in the three-way mix cover crop and 180 cm ¹⁵N burial depth were not included in the analysis since they were not represented in the late planting date treatment. The dependent variable was log10 ¹⁵N percent recovery.

To test if rye from spring sampling had higher ¹⁵N percent recovery than rye from fall sampling, an ANOVA was performed including the independent variables of cover crop sampling date and the interaction of sampling date x site. A separate analysis was performed for early-planted cover crops with ¹⁵N buried at 60 cm, early-planted cover crops with ¹⁵N buried at 120 cm, early-planted cover crops with ¹⁵N buried at 180 cm, late-planted cover crops with ¹⁵N buried at 60 cm, and late-planted cover crops with ¹⁵N buried at 120 cm. The dependent variable was log10 ¹⁵N percent recovery.

We evaluating if corn from experimental treatments contained higher at% ¹⁵N than background level. A one-sample t-test was performed to determine if 1) corn V5 and 2) corn grain samples had significantly higher at% ¹⁵N than the background level. We analyzed corn V5 and corn grain for the presence of at% ¹⁵N in plots from all early-planted cover crop treatments and all no cover crop control treatments.

We evaluated how much ¹⁵N buried tracer the corn captured. An ANOVA was performed for the experimental units with the ¹⁵N buried at 60 cm, to test if the corn V5 and corn grain ¹⁵N percent recovery was affected by the fixed effects of cover crop and the interaction of site x cover crop. A separate ANOVA was performed for the experimental units in the no cover crop control treatment, to test if the corn V5 and corn grain ¹⁵N percent recovery was affected by the fixed effects of ¹⁵N burial depth and the interaction of site x ¹⁵N burial depth.

Results

Experiment #1

Presence of buried ¹⁵N tracer in cover crops

The at% 15 N was significantly higher than the background level, at p < 0.001, in almost every treatment combination (67 out of 72 plots). Exceptions included two plots in which at% 15 N was significantly higher than the background level at p < 0.1, specifically, 1.) site two, rep two, late-planted radish root from 100 cm burial, and 2.) site 2, rep 2, early-planted, radish root from 200 cm burial, and three plots in which at% 15 N was not significantly higher than the background level, specifically, 1.) site two, rep three, early-planted rye from 200 cm burial, 2.) site one, rep one, early-planted rye from 200 cm burial, and 3.) site one, rep two, late-planted, radish root from 100 cm burial.

Percent recovery of buried ¹⁵N in cover crops

The mean ¹⁵N percent recovery of the cover crops ranged from 0.0076% - 35.8%.

Table 16 in **Appendix 6** list mean, standard deviation, minimum and maximum ¹⁵N percent recovery for every treatment combination. There was a significant difference in

the log10 15 N percent recovery among site x cover crop x planting date (p = 0.0594) and among planting date x 15 N burial depth (p < 0.0001) (Table 11). Radish versus rye cover crop species did not have different 15 N percent recovery regardless of planting date and site. Across cover crops, an early-planting date resulted in higher 15 N recovery from 100 cm deep than from 200 cm deep (p < 0.0001). However, a late-planting date did not result in higher 15 N recovery from 100 cm than from 200 cm (p = 0.9714). An early-planting date resulted in higher 15 N recovery from 100 cm than a late-planting date (p < 0.0001). However, an early-planting date did not result in higher 15 N recovery from 200 cm than a late-planting date (p = 0.2077). The early-planted rye had higher 15 N percent recovery than late-planted rye on site one (p = 0.0008) and site two (p = 0.0087). The early-planted radish had higher 15 N percent recovery than late-planted radish on site one (p < 0.0001), but not site two (p = 0.7402). The early-planted radish had higher 15 N percent recovery on site one than on site two (p = 0.0265). However, the late-planted radish and the early-and late-planted rye did not have differences in 15 N percent recovery between sites.

Soil sampling at ¹⁵N burial points

The soil at% ¹⁵N in the early-planted rye treatment plots that was buried at 100 cm appeared to move down the soil profile by December and May (Figure 11).

Experiment #2

Presence of buried ¹⁵N tracer in cover crops

In December, the at% 15 N was significantly (p < 0.01) higher than the background level for the radish shoot and root in 29 out of 30 plots and for rye in 30 out of 30 plots, regardless of planting-date or 15 N burial depth. In May, the rye at% 15 N was still significantly (p < 0.01) higher than the background level in 30 of 30 plots (Table 12).

In December, within the two-species cover crop, the at% 15 N in radish shoot, radish root, and rye always was significantly (p < 0.01) higher than the background where the 15 N was buried at 60 cm and in all but one plot where the 15 N was buried at 120 cm. Where the 15 N was buried at 180 cm, the at% 15 N in the radish and rye components of the cover crop were significantly (p < 0.05) higher than the background in five of six plots. By May, within the two-species cover crop, the rye had at% 15 N values significantly (p < 0.01) higher than the background in all six of the 60 cm 15 N burial plots, in five of the six 120 cm 15 N burial plots, and in two of the six 180 cm 15 N burial plots (Table 12).

By December, within the three-species cover crop, the radish shoot, radish root, rye, and clover (in all but one case) had at% 15 N values significantly (p < 0.01) higher than the background where the ¹⁵N was buried at 60 cm. Where the ¹⁵N was buried at 120 cm, the at% 15 N in the radish shoot, radish root, and rye was significantly (p < 0.05) higher than the background in every plot, while the clover had at% ¹⁵N values significantly (p < 0.05) higher than the background in six out of 12 plots. When the ¹⁵N was buried at 180 cm, the radish shoot, radish root, and rye had at % 15N significantly (p <0.01) higher than the background in five out of six plots, while the clover had at% ¹⁵N values significantly (p < 0.01) higher than the background in three out of six plots. By May, within the three-species cover crop, the rye and clover had at% ¹⁵N values significantly (p < 0.01) higher than the background in every plot where the ¹⁵N was buried at 60 cm. In the 120 cm 15 N burial plots, rye had at% 15 N values significantly (p <0.05) higher than the background in 10 out of 12 plots, while the clover had at% ¹⁵N values significantly (p < 0.05) higher than the background in eight out of 11 plots. In the 180 cm 15 N burial plots, rye had at% 15 N significantly (p < 0.1) higher than the

background in four out of six plots, while the clover had at% 15 N significantly (p < 0.01) higher than the background in three out of six plots (Table 12).

Percent recovery of buried ¹⁵N in cover crops

By December, within the early-planted cover crop treatment, there were significant (p < 0.0001) differences in the 15 N percent recovery among 15 N burial depths, with 60 cm burial > 120 cm burial > 180 cm burial. There was no difference among cover crops (p = 0.9897). There was no difference in the 15 N percent recovery between two-way mix and three-way mix cover crops for the 60 cm 15 N burial depth (p = 1.0000), 120 cm 15 N burial depth (p = 1.0000), or 180 cm 15 N burial depth (p = 1.0000). Within the late-planted cover crop treatment, there were significant (p < 0.0001) differences in the 15 N percent recovery between 15 N burial depth treatments, with 60 cm burial > 120 cm. There was no difference among cover crops (p = 0.308). By December, the early-planted cover crops had higher 15 N percent recovery than the late-planted cover crops (p < 0.0001).

For fall growth, early-planted cover crops captured on average 14.5% of the buried ¹⁵N from 60 cm, 2.67% of the buried ¹⁵N from 120 cm, and 0.31% of the buried ¹⁵N from 180 cm. Late-planted cover crops captured on average 1.36% of the buried ¹⁵N from 60 cm and 0.07% of the buried ¹⁵N from 120 cm (Figure 12).

Table 16 and Table 17 in **Appendix 6** list the mean, standard deviation, minimum and maximum ¹⁵N percent recovery for every treatment combination.

Cover crop sampling season differences

We assessed the rye ¹⁵N percent recovery for the December sampling versus the May sampling. For the early-planted rye, the percent recovery was higher in the fall than the spring for 60 cm, 120 cm, and 180 cm burial depths. For the late-planted rye, the

spring percent recovery (6.58%) was higher than the fall (1.45%) for the 60 cm burial, although not significantly (p = 0.1042), and the spring percent recovery (0.31%) was significantly (p = 0.0318) higher than the fall (0.09%) for the 120 cm burial, although by a very small amount (Table 13).

Presence of buried ¹⁵N in corn

In plots where no cover crop was planted, V5 corn had at% 15 N significantly (p < 0.1) above background (no 15 N application) level in four out of six replications when 15 N was buried at 60 cm, and three out of six replications when 15 N was buried at 120 cm and 180 cm. In plots where no cover crop was planted, corn grain had at% 15 N significantly (p < 0.05) above background (no 15 N application) level in four out of six replications when 15 N was buried at 60 cm, four out of six replications when 15 N was buried at 120 cm, and one out of six replications when 15 N was buried at 180 cm. In plots that had cover crops, V5 corn and corn grain always had at% 15 N significantly (p < 0.1) above background (no 15 N application) level, regardless of the cover crop species (Table 14).

In plots were no cover crop was planted, the 15 N percent recovery of V5 corn and corn grain was not different among 15 N burial depths. For the 60 cm 15 N burial depth, there were differences in V5 corn (but not corn grain) 15 N percent recovery among previous cover crop treatments. Because there was a significant (p = 0.0064) cover crop by site interaction, sites were analyzed separately. The distribution of the data was less skewed when sites were analyzed separately. At site four, the 15 N percent recovery of the V5 corn in the three-way mix treatment (0.399%) was greater than the V5 corn in the no cover crop control treatment (0.064%) (p = 0.0885). At site three, the 15 N percent recovery of the V5 corn in the three-way mix treatment (0.0989%) was greater than the

V5 corn in the no cover crop control treatment (0.0061%) (p = 0.0945). Although due to the design of the experiment and the sampling protocols these are all under estimates, in all cases, the amount of N that was traced through the V5 corn was less than 6% of the N taken up by the three-way mix cover crop.

Soil sampling at ¹⁵N burial points

Figure 13 depicts soil at% ¹⁵N in soil cores taken from plots that had no cover crop and ¹⁵N was buried at 60 cm deep. Soil cores were taken in February (site three) or April (site four) 2016, June 2016, and October 2016. Figure 14 depicts soil at% ¹⁵N in soil cores taken in February (site three) or April (site four) 2016 from plots that had no cover crop and ¹⁵N was buried at 120 cm deep.

Discussion

All species of cover crops, both early- and late-planted, contained levels of ¹⁵N higher than the background level, regardless of whether it was buried at 60 cm, 120 cm, or 180 cm. A few plots did not contain enriched ¹⁵N; however, these exception plots were not consistent between replications in a treatment. This finding was contrary to our hypothesizes, as we expected late-planted cover crops not to capture ¹⁵N from 120 cm or 180 cm. Levels of at% ¹⁵N above the background level indicate that the tissue contained ¹⁵N that originated from the buried tracer, either through the plant scavenging the tracer or the tracer being transferred between plants within a plot. The comparisons to see if the at% ¹⁵N is above the background are qualitative comparisons, not quantitative comparisons.

We performed quantitative comparisons between cover crop treatments by comparing cover crop ¹⁵N percent recovery. The ¹⁵N percent recovery values will be underestimates of the actual percent recover, as we had an unconfined system, and assumed there would be no ¹⁵N in plants greater than 20 cm from the burial point. Furthermore, we did not account for any ¹⁵N from the rye roots, clover roots, or radish fine roots. While we could not estimate the total plant recovery, the ¹⁵N percent recovery values that we did estimate allowed us to make relative comparisons between cover crops and planting dates, which was the main goal of the study.

In experiment #1, early-planted cover crops recovered on average 13.7% of the buried ¹⁵N in the 100 cm burial plots, 52 times more than the late-planted cover crops, which only recovered 0.26% of the buried ¹⁵N in the 100 cm burial plots. In experiment #2, the early-planted cover crops took up on average 14.5% of the ¹⁵N that was buried at 60 cm, while the late-planted cover crops took up only 1.4%. In other words, the earlyplanted cover crops took up 10 times more ¹⁵N than the late-planted cover crops from 60 cm deep. While the percent recovery of the buried ¹⁵N from 120 cm deep was small in all cases, the early-planted cover crops took up 38 times more ¹⁵N (2.67%) than the lateplanted cover crops (0.07%) from 120 cm deep. While the ¹⁵N percent recovery amounts from the 120+ cm burial depth were small, these findings support that radish and rye species both seem capable of quick, deep, root growth and are therefore promising "catch-crops". In a study at Rothamsted and at Woburn Experimental Farms in Bedfordshire that investigated winter wheat planting dates, Barraclough and Leigh (1984) found that for the September planting, roots were present 1 m deep by December, but for the October planting, roots did not reach 1 m until April. Furthermore, they found that

September-planted wheat had over four times as much root dry weight and root length by March than October-planted wheat (Barraclough and Leigh, 1984).

We expected that clover within the three-way mix would not be able to scavenge ¹⁵N from the deeper depths (120 cm, 180 cm). However, when ¹⁵N was buried at 120 cm, ¹⁵N was found in the clover tissue of 33% of the early-planted three-way mix replications and 67% of the late-planted three-way mix replications, and when ¹⁵N was buried at 180 cm, ¹⁵N was found in the clover tissue of 50% of the early-planted three-way mix replications. Because we did not have a monoculture clover treatment, we cannot know for certain if the clover was actually able to reach the deep buried ¹⁵N. However, we believe it is more likely that the clover picked up ¹⁵N from its deeper rooted neighbors (radish and rye), through sloughed cells, root turnover, or N leaching from senescing leaves (Dabney, et al., 2010; Maeght, et al., 2013; Smil, 1999).

We found no evidence that adding clover to the mix hindered the N uptake of radish or rye, a concern of some practitioners and policy makers. Both the radish and rye cover crops contained tracer from 180 cm deep regardless of whether they were part of a mixed stand with clover or not. Furthermore, there was no difference in the amount of ¹⁵N taken up by the two-way mix versus the three-way mix cover crops when ¹⁵N was buried at 60 cm, 120 cm, or 180 cm. Tosti and Thorup-Kristensen (2010) also found in a study using a mix of plant species with different colored roots found that the maximum root depth and depth penetration rate of beet was not affected by the presence of legumes.

There is a risk that if a cover crop does not scavenge deep soil NO₃ in the fall, the NO₃ would be out of reach for the spring cover crop. This is especially true in some environments with sandier soil or more precipitation, where NO₃ may leach rapidly

through the soil profile. In such environments, cover crops with fast growing roots may be the optimal system to capture NO₃ before it has a chance to leach to soil depths below the root zone. When we compared the percent recovery of the buried ¹⁵N in the fall rye growth versus spring growth, we found that the late-planted rye does take up additional ¹⁵N during spring growth, but not amounts that would be expected to have an impact on reducing NO₃ leaching or supplying N to a following crop.

We found evidence that corn following a cover crop was more likely to be enriched in ¹⁵N or have greater uptake of ¹⁵N than corn that did not follow a cover crop. However, the percent recoveries that we observed were very low (< 1%), regardless of whether there was a previous cover crop or not. Our study did not provide evidence that cover crops will recycle meaningful amounts of N from deep soil layers (60+ cm) to the subsequent corn. However, we believe this is a result of limitations of our experimental design. Specifically, we had an unconfined system, and therefore ¹⁵N may have moved horizontally outside of our sampling circle. We also did not account for ¹⁵N in the corn roots at the V5 sampling time, and we did not account for ¹⁵N in the corn roots or corn plant at the corn grain sampling time. Forage radish could provide N to the following corn crop, as it almost always winter-kills in Maryland and quickly decomposes, releasing mineral N into the soil surface layers (0-60cm) (Dean and Weil, 2009), although some studies have found that forage radish offers no N fertilizer replacement value (Ruark, et al., 2018). Crimson clover has been found to increase the following corn yield and corn N content (Kramberger, et al., 2009). Other studies provide evidence that mixed species or legume cover crops can increase subsequent corn yield (Marcillo and Miguez, 2017).

Conclusions and practical applications

Cover crops perform best if planted as early as possible. Our data show that early-September planting will allow cover crops to capture substantially more N than early-October planted cover crops. Thus, management practices such as shifting to earlier maturing corn hybrids and relay-crop establishment of cover crops should be evaluated. If planted by early-September, cover crops of radish, rye, and mixes of radish and rye (with or without crimson clover) were all effective at scavenging deep soil N. We found evidence that a late-planted rye cover crop will be able to scavenge additional N in the spring. Therefore, we recommend that rye cover crops be allowed to grow as much as possible in the spring to continue scavenging N. However, our findings from the ¹⁵N tracer in the soil profiles provide evidence that in some cases, nitrate is leaching from late-summer to spring, and therefore, rye will need to catch-up with the leaching N.

Table 7. Experiment #1, soil pH, percent sand, percent clay, percent C, percent N, NH₄-N (kg N ha⁻¹), and NO₃-N (kg N ha⁻¹) for each 20 cm soil depth increment (0-200 cm) and P, K, Mg, Ca, and S (mg kg⁻¹) for 0-30 cm soil. Reported pH, sand, clay, C, N, P, K, Mg, Ca, and S values are the average from three soil cores, one per block. Reported NO₃-N and NH₄-N values are the average from six soil cores, two per block.

	Soil depth	pН	Sand	Clay	Soil texture	C	N	NH ₄ -N	NO ₃ -N	P	K	Mg	Ca	S
	cm		9/	6		9,	6	kg N	ha ⁻¹		r	ng kg ⁻¹		
	0-20	5.52	85.4	2.9	Loamy fine sand	0.649	0.0340	20.5	35.3	95.3	49.0	37.3	269	8.00
	20-40	5.87	85.3	3.3	Loamy fine sand	0.288	BDL	9.14	21.6	56.0	43.7	29.0	203	4.67
	40-60	5.99	83.4	3.9	Loamy fine sand	0.143	BDL	5.49	13.7	1	1		1	
	60-80	6.13	85.4	4.3	Loamy fine sand	0.126	BDL	7.35	13.6	1	I	-	1	
Site one	80-100	6.35	82.8	6.1	Loamy fine sand	0.0846	BDL	3.85	6.15		-		1	
Site one	100-120	6.31	76.9	5.3	Loamy fine sand	0.0748	BDL	3.40	4.22		Ī		-	
	120-140	6.13	74.3	6.8	Sandy loam	0.0667	BDL	3.23	4.15	1	I	-	1	
	140-160	5.84	72.7	8.6	Sandy loam	0.0569	BDL	5.25	5.34	1	1	1	1	
	160-180	5.85	70.3	9.6	Sandy loam	0.0709	BDL	3.59	4.25				1	
	180-200	5.31	70.8	13.1	Sandy loam	0.0524	BDL	4.47	4.81	1	1	1	1	
	0-20	4.79	26.0	27.6	Clay loam	1.50	0.109	34.4	49.5	16.3	77.7	56.3	402	28.3
	20-40	4.71	20.0	37.1	Silty clay loam	0.780	0.0597	18.8	14.0	5.0	49.3	49.3	377	53.0
	40-60	4.27	12.1	53.5	Clay	0.345	0.0350	12.0	6.39					
	60-80	4.05	10.2	56.7	Clay	0.231	0.0297	10.3	5.13		-			
Site two	80-100	4.07	10.2	53.5	Clay	0.221	0.0247	8.87	4.40		1			
Site two	100-120	3.98	10.6	54.5	Clay	0.205	0.0273	8.96	4.13		-		-	
	120-140	3.99	10.4	53.5	Clay	0.171	BDL	7.77	3.34	1	1	1	1	
	140-160	3.98	12.9	47.2	Clay	0.157	BDL	7.13	3.05					
	160-180	4.01	12.6	46.4	Silty clay	0.149	BDL	6.53	2.79					
	180-200	4.02	11.0	51.0	Clay	0.158	BDL	6.86	2.95					

Table 8. Experiment #2, soil pH, percent sand, percent clay, percent C, percent N, NH₄-N (kg N ha⁻¹), and NO₃-N (kg N ha⁻¹) for each 15 cm soil depth increment (0-210 cm) and P, K, Mg, Ca, and S (mg kg⁻¹) for top three 15 cm soil depth increments (0-45 cm). Reported pH, sand, clay, C, N, P, K, Mg, Ca, and S values are the average from six cores (two cores 10 cm apart composited, taken in each of three blocks). Reported NO₃-N and NH₄-N values are the average from 15 cores (five cores in each of three blocks).

		pН	Sand	Clay	Soil texture	C	N	NH ₄ -N	NO ₃ -N	P	K	Mg	Ca	S
	Soil depth		9/	ó			%	kg N	ha ⁻¹		n	ng kg-1		
	0-15	6.14	77.0	3.4	Loamy fine sand	0.654	0.054	15.2	43.1	60.0	42.0	52.7	382	3.67
	15-30	6.29	74.6	5.5	Sandy loam	0.275	0.023	13.2	43.1	48.0	34.7	42.7	311	3.00
	30-45	6.48	65.6	8.2	Sandy loam	0.158	BDL	8.65	8.68	23.0	38.3	50.0	287	2.33
	45-60	6.52	65.6	9.5	Sandy loam	0.126	BDL	8.03	8.08			I		
	60-75	6.38	69.3	8.5	Sandy loam	0.103	BDL	9.13	12.2					
	75-90	6.38	75.0	6.1	Sandy loam	0.053	BDL	9.13	12.2					
Site three	90-105	6.32	79.1	6.9	Loamy fine sand	0.050	BDL	7.68	3.57					
Site tifree	105-120	5.95	75.6	9.7	Sandy loam	0.051	BDL	7.06	3.57					
	120-135	5.99	63.8	13.2	Sandy loam	0.098	BDL	8.65	8.54					
	135-150	5.85	68.5	12.5	Sandy loam	0.067	BDL	8.03	0.54					
	150-165	5.84	63.6	13.0	Sandy loam	0.038	BDL	9.58	3.95					
	165-180	5.46	57.5	14.7	Sandy loam	0.036	BDL		3.93					
	180-195	5.39	51.2	16.5	Loam	0.038	BDL							
	195-210	4.83	47.7	20.2	Loam	0.044	BDL]						
	0-15	6.05	64.8	6.7	Sandy loam	0.883	0.071	36.6	55.1	119.7	38.0	80.7	569	8.00
	15-30	6.09	61.4	8.9	Sandy loam	0.368	0.032			56.3	26.3	44.7	389	4.67
	30-45	6.05	58.2	12.1	Sandy loam	0.154	BDL	24.5	10.4	4.0	27.3	35.0	365	14.7
Site four	45-60	5.50	55.3	15.9	Sandy loam	0.103	BDL							
	60-75	5.06	47.4	18.0	Loam	0.134	BDL	24.7	8.99					
	75-90	4.75	41.1	18.0	Loam	0.074	BDL							
	90-105	4.34	39.9	20.8	Loam	0.083	BDL	24.5	12.2					

105-120	4.20	27.7	21.9	Silt loam	0.073	BDL			 	 	
120-135	4.23	28.2	21.5	Silt loam	0.095	BDL	28.4	16.0	 	 	
135-150	4.17	26.1	19.8	Silt loam	0.068	BDL			 	 	
150-165	4.20	25.9	18.3	Silt loam	0.058	BDL	28.0	14.9	 	 	
165-180	4.07	25.9	17.8	Silt loam	0.064	BDL			 	 	
180-195	4.12	34.3	18.0	Loam	0.066	BDL			 	 	
195-210	4.07	27.9	16.5	Silt loam	0.058	BDL]		 	 	

Table 9. Experiment #2, experimental treatment combinations. Experimental factors that defined the treatments included cover crop, cover crop planting date, and ¹⁵N burial depth. The cover corps indicated in white were only planted early, not late. The cover crops indicated in grey were planted early and late.

crops marcated in	grey were planted
Cover crop	¹⁵ N burial depth
Radish	60 cm
Radish	120 cm
Radish	180 cm
Radish	No ¹⁵ N
Rye	60 cm
Rye	120 cm
Rye	180 cm
Rye	No ¹⁵ N
Two-way mix	60 cm
Two-way mix	120 cm
Two-way mix	180 cm
Two-way mix	No ¹⁵ N
Three-way mix	60 cm
Three-way mix	120 cm
Three-way mix	180 cm
Three-way mix	No ¹⁵ N
No cover crop	60 cm
No cover crop	120 cm
No cover crop	180 cm
No cover crop	No ¹⁵ N

Table 10. Experiment #2, soil samples taken (and depths analyzed in parentheses) per block. The number of asterisks indicate the number of composite cores per sample. Two cores from the same distance from the tracer (dist ¹⁵N) were from two different burial points within a plot; two cores from different dist ¹⁵N were from one burial point within the plot.

	60 cm ¹⁵ N depth	1	120 cm ¹⁵ N depth			
	10 cm dist ¹⁵ N	20 cm dist ¹⁵ N	10 cm dist ¹⁵ N	20 cm dist ¹⁵ N		
Feb (site three)/	*	*	*	*		
Apr (site four)	(30-120 cm)	(30-120 cm)	(60-2)	10 cm)		
	**	**				
Jun	(45-210 cm)	(45-210 cm)	-	-		
	**					
Oct	(45-210 cm)	-	-	-		

Table 11. Experiment #1, analysis of variance (ANOVA) tests of fixed effects for log10 ¹⁵N percent recovery

Experimental factor	p-value
Cover crop	0.044
Planting date	0.015
¹⁵ N burial depth	< 0.0001
Cover crop x Planting date	0.57
Planting date x ¹⁵ N burial	< 0.0001
Cover crop x ¹⁵ N burial	0.97
Cover crop x Planting date x ¹⁵ N burial	0.76
Site x Planting date	0.013
Site x Cover crop	0.16
Site x ¹⁵ N burial	0.56
Site x Cover crop x Planting date	0.058
Site x Planting date x ¹⁵ N burial	0.099
Site x Cover crop x ¹⁵ N burial	0.88
Site x Planting date x Cover crop x ¹⁵ N burial	0.49

Table 12. Experiment #2, percent of the six replications within a given treatment with cover crop tissue type at% 15 N significantly (p < 0.01) above background (no 15 N

application) level.

cover crop	Planting date	radish shoot	radish root	rye s	shoot	clove	r shoot		
		fall	fall	fall	spring	fall	spring		
		60 cm ¹⁵ N burial							
		Replications with at% ¹⁵ N above background (%)							
	Early-planted	100	100						
Radish	Late-planted	100	100	I		-			
	Early-planted			100	100				
Rye	Late-planted			100	100	-			
Two-way mix	Early-planted	100	100	100	100				
Three-way	Early-planted	100	100	100	100	100	100		
mix	Late-planted	100	100	100	100	83	100		
					⁵ N burial				
				h at% ¹⁵	N above l	oackgrou	und (%)		
	Early-planted	100	100						
Radish	Late-planted	83	83						
	Early-planted			100	100	-			
Rye	Late-planted			100	100	1			
Two-way mix	Early-planted	83	100	100	83				
Three-way	Early-planted	100	100	100	67	33	100		
mix	Late-planted	100*	100	100	100*	67*	50*		
					⁵ N burial				
		Replica	ations wit	h at% ¹⁵	N above l	oackgrou	und (%)		
	Early-planted	100	100						
Radish	Late-planted								
	Early-planted			100	100				
Rye	Late-planted								
Two-way mix	Early-planted	67 [†]	83*	83	33				
Three-way	Early-planted	83	83	83	67 [†]	50	50		
mix	Late-planted								
* $p < 0.05$ † $p < 0.1$									

Table 13. Experiment #2, ¹⁵N percent recovery for fall and spring rye. The p-values indicated differences between fall and spring log10 ¹⁵N percent recovery.

	¹⁵ N burial	Fall ¹⁵ N percent	Spring ¹⁵ N percent	p-value
Cover crop planting date	depth	recovery	recovery	p-value
	60 cm	16.07%	6.94%	0.30
Early-planting	120 cm	1.22%	0.78%	0.11
	180 cm	0.19%	0.10%	0.036
Late plenting	60 cm	1.45%	6.58%	0.10
Late-planting	120 cm	0.09%	0.31%	0.032

Table 14. Experiment #2, percent of the six replications within a given treatment with V5 corn or corn grain at% 15 N significantly (p < 0.01) above background (no 15 N application) level.

Previous cover crop	V5 corn	Corn grain
	60 cm ¹	⁵ N burial
Early-planted, radish	100	100
Early-planted, rye	100*	100
Early-planted, two-way mix	100 [†]	100
Early-planted, three-way mix	100	100
No cover crop	67 [†]	67
	120 cm	¹⁵ N burial
No cover crop	50^{\dagger}	67
	180 cm	¹⁵ N burial
No cover crop	50 [†]	17*
* p < 0.05	•	•
$\dagger p < 0.1$		

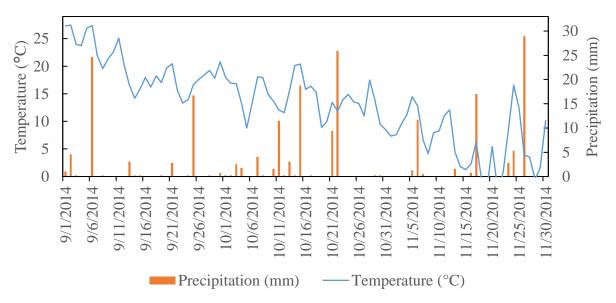


Figure 7. Experiment #1, temperature ($^{\circ}$ C) and precipitation (mm) from 1 Sep 2014 to 30 Nov 2014.

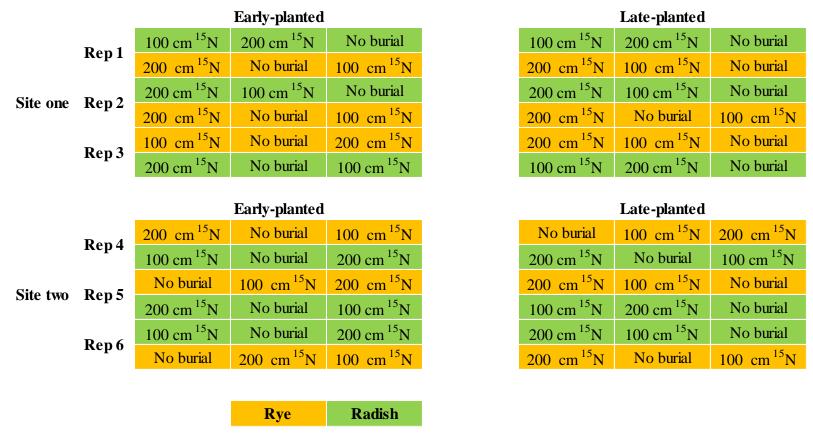


Figure 8. Experiment #1, split-split plot experimental design and treatments, showing cover crop planting date as the main plot factor, cover crop as the split-plot factor, and ¹⁵N burial depth as the split-split plot factor.

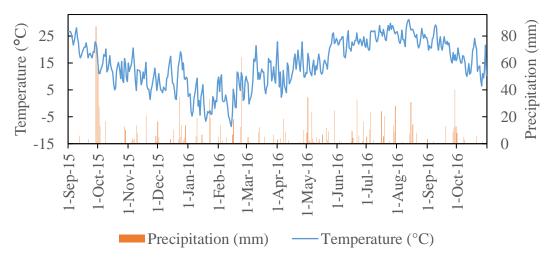


Figure 9. Experiment #2, temperature (°C) and precipitation (mm) from 3 Sep 2015 to 7 Oct 2016.

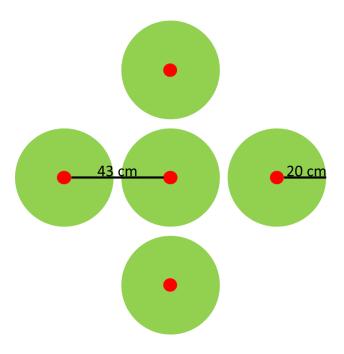


Figure 10. Experiment #2, horizontal spatial arrangement of ¹⁵N burial holes (red dots) and areas around burial points in which biomass was sampled (green circles).

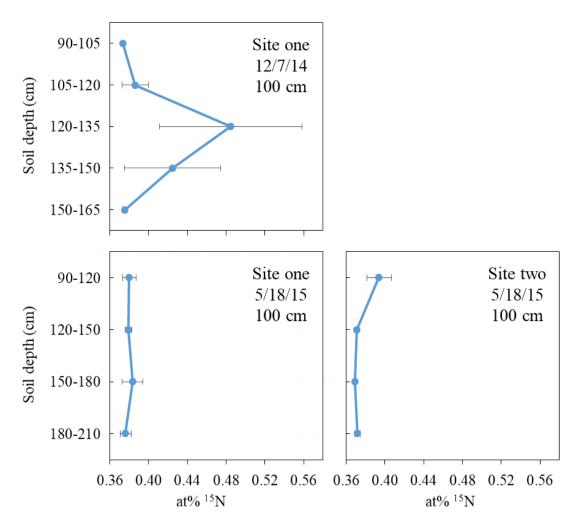


Figure 11. Experiment #1, soil at% ¹⁵N in soil cores taken in December 2014 (site one) and May 2015 (sites one and two) from early-planted rye plots in which ¹⁵N was buried at 100 cm. The at% ¹⁵N values are the average of two blocks in December and three blocks in May.

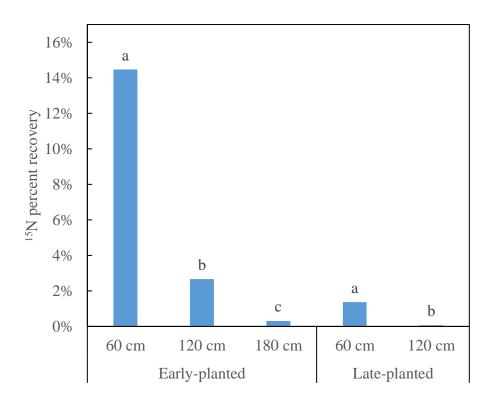


Figure 12. Experiment #2, December 15 N percent recovery from each 15 N burial depth for early- and late-planted cover crops (across all cover crops). The 15 N burial depth values for log10 15 N percent recovery within the same cover crop planting date treatment followed by the different letters are significantly different (p < 0.05).

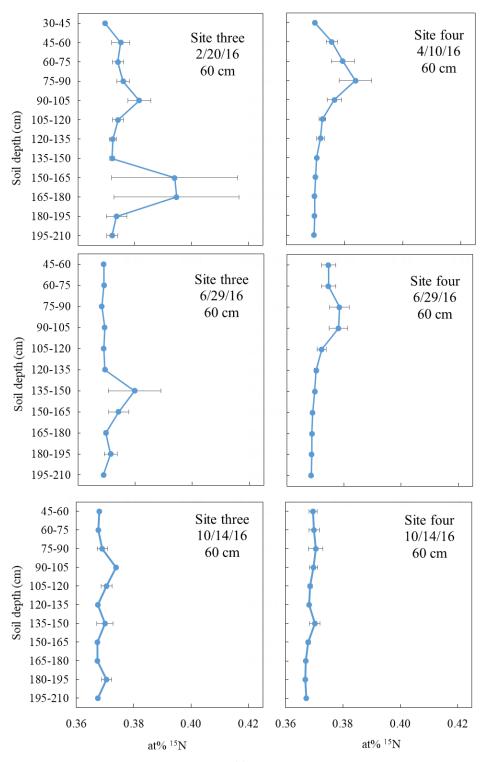


Figure 13. Experiment #2, soil at% ¹⁵N in soil cores taken in February 2016 (site three) or April 2016 (site four), June 2016 (sites three and four), and October 2016 (sites three and four) from the no cover crop control plots in which ¹⁵N was buried at 60 cm. The at% ¹⁵N values are the average of three blocks.

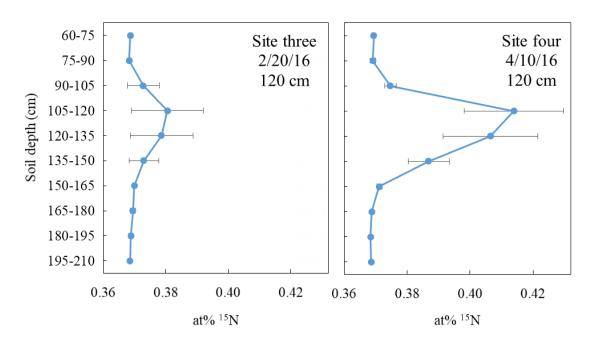


Figure 14. Experiment #2, soil at% ¹⁵N in soil cores taken in February 2016 (site three) or April 2016 (site four) from the no cover crop control plots in which ¹⁵N was buried at 120 cm. The at% ¹⁵N values are the average of three blocks.

Appendix 5. Detailed methods for estimation of rye biomass

Table 15. Samples taken to estimate rye biomass, the number of samples (N) taken per treatment, regression equations relating rye

patchiness, height, and/or percent cover to biomass, and adjusted R² for each regression equation

Sample	Treatment	Samples taken	N	Equation	Adj R ²
7 Jan	Fall rye	At site 3 and site 4, five samples were taken in early-planting plots, and five in lateplanting plots	20	Dry matter = 0.91078*(height) – 0.47720*(patchiness) + 0.24967*(percent cover)	0.95
2016	Fall two-way mix and three-way mix	At site 3 and site 4, five samples were taken in early-planting plots, and five in lateplanting plots	20	dry matter = 0.42022*(percent cover ¹)	0.91
O Mary	Spring rye	At site 3 and site 4, three samples were taken in early-planting plots, and three in late-planting plots	12	dry matter = 0.29445*(height) + 1.40593*(percent cover)	0.87
8 May 2016	Spring two-way mix and three-way mix	At site 3 and site 4, four samples were taken in two-way mix plots, five in early-planting three-way mix, and five in late-planting three-way mix	28	dry matter = 0.04924*(height) + 0.66463*(patchiness) + 1.07426*(percent cover)	0.89

¹ We used only the percent cover independent variable for the fall rye in mixed cover crop treatments. Fall samples were not taken in two-way mix or three-way mix treatments; therefore we needed to use fall samples from rye treatments. Rye growth within rye treatment was shorter than rye growth in mixed treatments, likely because rye in mixed treatments was growing tall to compete for sunlight with the radish. Therefore, using the rye height variable resulted in what visually appeared to be unrealistically high values for biomass. Including the variable of patchiness also resulted in what visually appeared to be unrealistically high values for biomass. Rye percent cover alone resulted in what visually appeared to be realistic estimates for biomass.

Appendix 6. ¹⁵N percent recovery data

Table 16. Percent recovery of ¹⁵N for December sampled radish, rye, two-way mix (2mix), and three-way mix (3mix) cover crops, showing the number of observations per reported value (N), mean, standard deviation (SD), minimum value (Min) and maximum value (Max)

Exp. #	Site	Planting date	Cover crop	N	Mean	SD	Min	Max			
			100 cm ¹⁵ N	buri	al			1			
Exp. #1	Site 2	Early	Radish	3	4.68%	0.0622	0.55%	11.8%			
Exp. #1	Site 1	Early	Radish	3	35.8%	0.158	22.3%	53.2%			
Exp. #1	Site 2	Early	Rye	3	3.32%	0.0273	0.46%	5.89%			
Exp. #1	Site 1	Early	Rye	3	10.9%	0.109	0.50%	22.3%			
Exp. #1	Site 2	Late	Radish	3	0.97%	0.016172	0.01%	2.84%			
Exp. #1	Site 1	Late	Radish	3	0.03%	0.000093	0.02%	0.04%			
Exp. #1	Site 2	Late	Rye	3	0.05%	0.000605	0.01%	0.12%			
Exp. #1	Site 1	Late	Rye	3	0.01%	0.000029	0.00%	0.01%			
200 cm ¹⁵ N burial											
Exp. #1	Site 2	Early	Radish	3	0.05%	0.000234	0.03%	0.07%			
Exp. #1	Site 1	Early	Radish	3	0.64%	0.00460	0.34%	1.17%			
Exp. #1	Site 2	Early	Rye	3	0.10%	0.00141	0.00%	0.26%			
Exp. #1	Site 1	Early	Rye	3	0.07%	0.000525	0.01%	0.11%			
Exp. #1	Site 2	Late	Radish	3	0.09%	0.000404	0.05%	0.13%			
Exp. #1	Site 1	Late	Radish	3	0.07%	0.000174	0.05%	0.08%			
Exp. #1	Site 2	Late	Rye	3	0.02%	0.000260	0.01%	0.05%			
Exp. #1	Site 1	Late	Rye	3	0.03%	0.000196	0.01%	0.05%			
			60 cm ¹⁵ N b	ouria	ıl						
Exp. #2	Site 4	Early	2mix	3	18.1%	0.0444	13.0%	21.4%			
Exp. #2	Site 3	Early	2mix	3	10.6%	0.0352	7.27%	14.3%			
Exp. #2	Site 4	Early	3mix	3	15.7%	0.0452	12.2%	20.8%			
Exp. #2	Site 3	Early	3mix	3	7.68%	0.0435	3.16%	11.8%			
Exp. #2	Site 4	Early	Radish	3	19.0%	0.0391	14.5%	21.4%			
Exp. #2	Site 3	Early	Radish	3	12.4%	0.130	1.53%	26.9%			
Exp. #2	Site 4	Early	Rye	3	26.3%	0.211	2.98%	43.9%			
Exp. #2	Site 3	Early	Rye	3	5.81%	0.0572	2.06%	12.4%			
Exp. #2	Site 4	Late	3mix	3	0.60%	0.00230	0.46%	0.87%			
Exp. #2	Site 3	Late	3mix	3	0.27%	0.00219	0.14%	0.52%			
Exp. #2	Site 4	Late	Radish	3	1.91%	0.0177	0.12%	3.67%			
Exp. #2	Site 3	Late	Radish	3	2.48%	0.0256	0.50%	5.37%			
Exp. #2	Site 4	Late	Rye	3	2.24%	0.0282	0.34%	5.48%			

Exp. #2	Site 3	Late	Rye	3	0.67%	0.00609	0.03%	1.25%
			120 cm ¹⁵ N	buri	al			
Exp. #2	Site 4	Early	2mix	3	1.53%	0.0108	0.30%	2.26%
Exp. #2	Site 3	Early	2mix	3	6.34%	0.106	0.12%	18.5%
Exp. #2	Site 4	Early	3mix	3	1.41%	0.00408	0.98%	1.80%
Exp. #2	Site 3	Early	3mix	3	5.26%	0.0727	0.16%	13.6%
Exp. #2	Site 4	Early	Radish	3	0.41%	0.00207	0.28%	0.65%
Exp. #2	Site 3	Early	Radish	3	3.95%	0.0479	0.03%	9.30%
Exp. #2	Site 4	Early	Rye	3	1.13%	0.00741	0.43%	1.91%
Exp. #2	Site 3	Early	Rye	3	1.31%	0.00597	0.68%	1.87%
Exp. #2	Site 4	Late	3mix	3	0.07%	0.000257	0.04%	0.08%
Exp. #2	Site 3	Late	3mix	3	0.05%	0.000405	0.01%	0.09%
Exp. #2	Site 4	Late	Radish	3	0.04%	0.000333	0.00%	0.06%
Exp. #2	Site 3	Late	Radish	3	0.07%	0.000607	0.03%	0.14%
Exp. #2	Site 4	Late	Rye	3	0.13%	0.000894	0.03%	0.20%
Exp. #2	Site 3	Late	Rye	3	0.06%	0.000332	0.02%	0.08%
			180 cm ¹⁵ N	buri	al			
Exp. #2	Site 4	Early	2mix	3	0.12%	0.000479	0.07%	0.16%
Exp. #2	Site 3	Early	2mix	3	0.48%	0.00539	0.02%	1.08%
Exp. #2	Site 4	Early	3mix	3	0.32%	0.00377	0.01%	0.74%
Exp. #2	Site 3	Early	3mix	3	0.22%	0.00226	0.08%	0.48%
Exp. #2	Site 4	Early	Radish	3	0.29%	0.00229	0.07%	0.53%
Exp. #2	Site 3	Early	Radish	3	0.65%	0.00491	0.25%	1.20%
Exp. #2	Site 4	Early	Rye	3	0.24%	0.000795	0.16%	0.32%
Exp. #2	Site 3	Early	Rye	3	0.15%	0.00102	0.07%	0.26%

Table 17. Percent recovery of ¹⁵N for April sampled two-way mix (2mix), three-way mix (3mix), and rye cover crops, showing the number of observations per reported value (N), mean, standard deviation (SD), minimum value (Min) and maximum value (max)

Site	Planting date	Cover crop	N	Mean	SD	Min	Max			
		60 cm	¹⁵ N	burial						
Site 4	Early	2mix	3	2.73%	0.0236	0.90%	5.40%			
Site 3	Early	2mix	3	0.97%	0.00193	0.78%	1.16%			
Site 4	Early	3mix	3	2.02%	0.0131	0.68%	3.29%			
Site 3	Early	3mix	3	0.63%	0.00292	0.30%	0.81%			
Site 4	Early	Rye	3	11.2%	0.0222	9.40%	13.7%			
Site 3	Early	Rye	3	2.68%	0.0171	1.65%	4.65%			
Site 4	Late	3mix	3	5.80%	0.0540	2.50%	12.0%			
Site 3	Late	3mix	3	0.14%	0.000551	0.08%	0.19%			
Site 4	Late	Rye	3	12.5%	0.104	0.74%	20.5%			
Site 3	Late	Rye	3	0.68%	0.00672	0.05%	1.39%			
120 cm ¹⁵ N burial										
Site 4	Early	2mix	3	0.11%	0.000798	0.02%	0.16%			
Site 3	Early	2mix	3	1.20%	0.0196	0.07%	3.46%			
Site 4	Early	3mix	3	0.16%	0.00197	0.04%	0.38%			
Site 3	Early	3mix	3	0.62%	0.00874	0.03%	1.62%			
Site 4	Early	Rye	3	0.54%	0.000639	0.48%	0.61%			
Site 3	Early	Rye	3	1.02%	0.00947	0.39%	2.11%			
Site 4	Late	3mix	3	0.31%	0.00308	-0.04%	0.53%			
Site 3	Late	3mix	3	0.37%	0.00512	0.04%	0.96%			
Site 4	Late	Rye	3	0.47%	0.00300	0.28%	0.81%			
Site 3	Late	Rye	3	0.15%	0.000499	0.09%	0.19%			
		180 cm	¹⁵ N	burial						
Site 4	Early	2mix	3	0.04%	0.000240	0.02%	0.07%			
Site 3	Early	2mix	3	0.09%	0.00129	0.00%	0.23%			
Site 4	Early	3mix	3	0.03%	0.000387	0.00%	0.07%			
Site 3	Early	3mix	3	0.02%	0.000115	0.01%	0.03%			
Site 4	Early	Rye	3	0.12%	0.000559	0.08%	0.19%			
Site 3	Early	Rye	3	0.07%	0.000338	0.05%	0.11%			

Chapter 4: Cover crop systems influence on deep soil N dynamics and the following corn crop: on-farm investigations

Abstract

In the Mid-Atlantic USA, substantial mineral N (100-500 kg N ha⁻¹) remains in the 0-2 m soil in September, 78% deeper than 30 cm, which is at risk to leach over winter months. We hypothesized that deep-rooted cover crops planted by early-September could capture residual N, and potentially recycle this N for following cash crops. We performed experiments on 19 farms in Maryland and Pennsylvania investigating the effects of four cover crop systems (forage radish, winter cereal, forage radish + winter cereal + crimson clover, no cover crop control) on cover crop biomass, N uptake, and inorganic N distribution within the upper 210 cm of soil in late-fall and early-spring, and the following corn crop's growth and yield. In late-fall, radish reduced soil NO₃-N to 90 cm deep, while winter cereal or mix cover crops reduced NO₃-N to 60 cm deep. In the spring, radish released NO₃-N on the soil surface (0-30 cm), but was less effective than winter cereal at reducing NO₃ from 30-150 cm deep. Winter cereal was the most effective at reducing soil NO₃ throughout the entire soil profile. Mix was more effective than winter cereal and as effective as radish at ensuring available NO₃ on the soil surface (0-30 cm), and was as effective as winter cereal in reducing soil NO₃ from 30-210 cm soil. The V5 corn biomass and N content were affected by the previous cover crop treatment in the order radish > mix = control > winter cereal. At the farmers' standard N fertilizer application rate, corn yield following radish or control was higher than winter cereal, and corn yield following radish was higher than mix. Cover crops can be fit within the

framework of existing crop systems to scavenge and accumulate N, and through their decomposition supply N for subsequent crops, therein improving the overall N use efficiency of the cropping system.

Introduction

Cropping systems, weather patterns, and soil characteristics in the Mid-Atlantic USA make agricultural systems in this region prone to nitrate (NO₃) leaching. A common cropping system in Maryland is a corn (Zea mays L.) to soybean (Glycine max (L.) Merr.) rotation. In this rotation, from September to May there is no crop actively taking up nitrogen (N) from the soil. In addition, soybean acquires 50-60% of its N through symbiotic N fixation, and therefore, does not scavenge N from the soil profile as efficiently as non-legume crops (Salvagiotti, et al., 2008). The region has a humid climate with annual rainfall relatively uniformly distributed throughout the entire year. The average annual precipitation for Beltsville, MD is 1063 mm (US Climate Data, 2018). Therefore, in the case of the typical corn/soybean rotation, the majority of the rainfall is occurring when there is no crop growing and taking up N or water from the soil profile. Many soils of the Coastal Plain physiographic region surrounding the Chesapeake Bay have sandy textures, through which NO₃ leaches more rapidly than through the finer textured soils of the Piedmont and other areas with soils formed from metamorphic and sedimentary rock.

Plants grown in the fall, following harvest of the cash crop, are called cover crops, catch crops, or green manures (Thorup-Kristensen, et al., 2003). Some cover crop species have the potential to quickly grow deep roots, and could serve as a "catch crop" to

capture NO₃ in the fall months before it leaches out of reach, and potentially release N in the spring months to be used by the following cash crop (Dabney, et al., 2010; Meisinger, et al., 1990; Meisinger, et al., 1991). For example, while corn roots did not reach depths > 1.2 m, subsequent winter wheat could use soil NO₃ up to 2 m deep (Zhou, et al., 2008). Cover crops can serve to reduce NO₃ concentration in aquifers used for drinking water and to decrease NO₃ concentrations in surface waters, lessening the risk of eutrophication and associated negative environmental effects (Thorup-Kristensen, et al., 2003). Cover crops can be fit within the framework of the existing crop system to scavenge and accumulate N in their tissue, and then through their decomposition, to supply N for subsequent crops. Such a cover crop system would improve the overall N use efficiency of the cropping system.

To capture N before it leaches out of reach, it is important to plant cover crops as soon as possible after cash crops and also to use deep-rooted, fast-growing species. Cover crops must capture NO₃ that is progressively moving deeper through the soil profile after cash crops stop taking up N. The deeper N is in the soil profile, the more likely it is to leach from the soil, and therefore it is particularly important for cover crops to scavenge deep soil N.

Nitrogen that is captured by cover crops can be a valuable resource for farmers, if it is released into the soil as available N in synchrony with cash crop N uptake needs (Dabney, et al., 2001). However, cover crops can have detrimental effects on the environment or agronomic system if cover crop N mineralization leads to increased N leaching, or if cover crop N immobilization leads to increased fertilizer use on crops. Furthermore, unlike amending the soil with manure or compost, non-legume cover crops

do not add N to the soil, but rather capture N from the soil and then return the N back to the soil (Thorup-Kristensen, et al., 2003). Preemptive competition can occur, if a cover crop takes up N that would have remained in the rooting zone of the subsequent crop in the absence of the cover crop (Thorup-Kristensen, et al., 2003). While residue mineralization will affect mostly the soil surface layers, which would affect the main crop early in the growing season, preemptive competition of N resources can reduce subsoil N, which could adversely affect the main crop later in the growing season (Thorup-Kristensen, 1993). The apparent effect of cover crops will depend on the soil depth considered (e.g., examining 0-50 cm may result in very different conclusions than examining 0-150 cm). To minimize negative preemptive competition effects, the expected leaching intensity of the field and the rooting depth of the subsequent crop should be considered (Thorup-Kristensen and Nielsen, 1998).

The long-term goal of using cover crops is to sustain higher levels of production with less N loss, and therefore, the efficacy of cover crops may largely depend on choosing appropriate species according to the local hydrologic regime and minimizing preemptive competition (Thorup-Kristensen, et al., 2003). For example, Thorup-Kristensen (2006a) observed that, in the spring, the subsoil (1-2.5 m) contained 120 kg N ha⁻¹ where no cover crop had been grown but only 49 and 60 kg ha⁻¹, respectively, where radish (*Raphanus sativus* L. var. *oleiformis*) and Italian ryegrass (*Lolium multiflorum* Lam.) cover crops had been grown. During the following crop season, they measured the available inorganic N in the root zone for each crop and the actual N uptake by each crop. They found that there was more available N and N uptake for leek (*Allium porrum* L.) after radish and leek after ryegrass in comparison to leek after no cover crop, and they

found there was more available N and N uptake for beet (*Beta vulgaris* L. var. *esculenta* L.) after ryegrass (they did not investigate beet after radish) in comparison to beet after no cover crop. However, the N uptake for white cabbage was decreased following ryegrass or forage radish cover crop (Thorup-Kristensen, 2006a).

In the current study, we investigated the biomass N uptake, soil inorganic N depletion, and corn response following various deep-rooted cover crop systems on a broad range of soil types, geographic areas, and management regimes in Maryland and southeast Pennsylvania. Specifically, we investigated the effects of four cover crop systems—1) forage radish monoculture, 2) winter cereal monoculture, 3) winter cereal + forage radish + legume mixture, 4) no cover crop control on:

- 1) Inorganic N distribution within the upper 210 cm of soil in late-fall and early-spring;
- 2) Cover crop biomass and N uptake
- 3) Corn biomass and N content, and soil (0-30 cm) NO₃-N and NH₄-N concentration in June (corn growth stage V5);
- 4) Corn yield, with various N fertilizer rates.

Materials and Methods

Locations

Cover crop experiments were conducted on 19 farm sites, two of which were at a University of Maryland dairy farm (Central Maryland Research and Education Center, Clarksville, MD) with the other 17 being on private commercial farms. Experiments were located throughout the main agricultural areas in Maryland and Southern Pennsylvania.

Appendix 7, Table 27 lists soil characteristics including pH, percent sand, percent clay, percent C, and percent N of the study site soils for each 30 cm depth increment from 0-210 cm, and percent soil organic matter (SOM), and P, K, Mg, Ca, and S (mg kg⁻¹) for 0-30 cm.

Cover crop experimental design and treatments

Depending on the farmers' preferences, situation and facilities, the cover crop experiments varied somewhat among sites, with regard to plot size, specific cereal species used, tillage practices and planting dates. In general, the experiments followed a randomized complete block design with three to four blocks. Plot size was dependent on the equipment and land available on a given farm, and were on average 409 m², ranging from 45 m² to 2128 m² (Table 19). Cover crop treatments typically included 1) forage radish (radish), 2) winter cereal (cereal), 3) a multi-species cover crop comprised of forage radish + winter cereal + crimson clover (*Trifolium incarnatum* L.) (mix), and 4) a control of winter weeds only with no-cover crop planted (control). The winter cereal species and species in the cover crop mix varied according to farmer preference.

Lancaster IB and Kent II farms also had a late-planted cover crop mix treatment, which was planted 2-4 weeks after the other cover crops. Table 18 indicates site histories of the cover crop experiment sites. Table 19 indicates cover crop treatments, planting dates, management details, and weather details.

Corn response experimental design and treatments

On nine of the farms with cover crop experiments, corn was planted following cover crop termination to test for cover crop effects on V5 corn growth and/or corn yield.

On five farms the fall cover crop treatment main plots were split, at corn planting, into

multiple N fertilizer rate sub plots (Table 20), and the response of corn to N fertilization was measured. Table 20 describes the corn planting, N fertilization, herbicide, harvest, and sampling regime in each experiment.

Biomass and soil sampling and analysis

Cover crop biomass samples and soil cores from 0-210 cm deep were obtained in late-fall, prior to the cover crop species dying or becoming dormant for the winter, and in late-spring, shortly before cover crop termination.

Biomass was collected from two to five 0.25 m² quadrats per plot (**Appendix 8**, Table 28). Quadrats were randomly placed in a plot, approximately equal distance apart, one near each end and the others near the middle. No samples were taken within one meter of the plot boundaries to avoid edge effects. Winter cereal species were harvested 1 cm above the soil surface. Forage radish was harvested by hand-pulling the fleshy root from the soil. The radish leaf and root were separated. Radish roots were thoroughly washed to remove all soil. In the mixture cover crop plots, the radish, winter cereal, and legume species were separated. The legume was harvested 1 cm above the soil surface. For all treatments, weeds were separated from cover crops. Weeds were only collected if they were estimated to be > 5% of the total cover crop biomass. In the lab, radish roots were chopped into approximately 2 cm³ pieces to expedite drying. Biomass samples were dried in paper bags at 45 °C until a constant weight was attained. Dry biomass weights were recorded. The biomass was ground to 1 mm sieve size. Biomass samples were analyzed for total C and N at University of Maryland Department of Environmental Science and Technology Analytical Lab (LECO CN628 Elemental Analyzer LECO Corp., St. Joseph, MI).

The biomass of each tissue type (radish shoot, radish root, winter cereal shoot, legume) was converted to kilograms per hectare (**Equation 15**). Cover crop biomass was multiplied by the percent N to get the cover crop N content, and the amount of N taken up by cover crops was converted to kilograms per hectare (**Equation 16**).

For radish and mixture treatments, the biomass and the N content of the tissue types were summed to determine the cover crop biomass per hectare. Weeds were not included in the biomass calculation if they were < 5% of the total biomass in a quadrat.

Equation 15. Cover crop biomass per hecatare

$$\frac{\text{kg biomass per species}}{\text{ha}} = \left(\frac{\text{g biomass}_{\text{Quad 1}}}{0.25 \text{ m}^2} + \frac{\text{g biomass}_{\text{Quad 2}}}{0.25 \text{ m}^2}\right) * \frac{10,000 \text{m}^2}{1 \text{ ha}} * \frac{1 \text{ kg}}{1000 \text{ g}}$$

Equation 16 Cover crop N per hectare

$$\frac{kg \ N \ per \ species}{ha} = \left(\frac{(g \ biomass*\frac{\% \ N}{100})_{Quad \ 1}}{0.25 \ m^2} + \frac{(g \ biomass*\frac{\% \ N}{100})_{Quad \ 2}}{0.25 \ m^2}\right) * \frac{10,000 \ m^2}{1 \ ha} * \frac{1 \ kg}{1000 \ g}$$

Growing degree days (GDD) and precipitation available to cover crops were

determined between cover crop planting date and fall or spring cover crop sampling, based on the precipitation and temperature data from the closest weather station to the study site (**Appendix 9**, Table 29). Growing degree days were calculated using **Equation 17**. The base temperature used was 4.4°C. While the base temperature of 10°C is typically used for corn growth (http://www.cpc.ncep.noaa.gov/products/analysis_monitoring/cdus/degree_days/gdd.sht ml), lower temperatures are often used when studying cover crop growth—e.g., 4.4°C by Mirsky, et al. (2011), 5°C by Komainda, et al. (2016) and Schroder, et al. (1996), and 0°C by Farsad, et al. (2011) and Tribouillois, et al. (2016).

Equation 17 Growing degree days

 $GDD = \sum_{P\text{-}S} ((daily\ max\ temperature\ (^{\circ}C) + daily\ min\ temperature)/2) - 4.4^{\circ}C$ Where, P = planting date, and S = sampling date

Soil cores were taken by hand-driving a Veihmeyer probe (Veihmeyer, 1929) into the ground using a 6.8 kg drop hammer. Cores were taken from 0 to 210 cm deep when possible, or until the probe reached groundwater or an impenetrable layer of rock. After the probe was driven into the ground, a jack and lever system was used to remove the probe from the ground.

In the experiments conducted in the 2014-2015 season, two soil cores were taken per plot. In the fall, the two cores were taken approximately 60 cm apart, and in the spring the two cores were taken on opposite ends of the plot. Soil was extracted in two increments, from 0-120 cm deep, and from 120-210 cm deep. Soil cores were emptied into a trough and arranged, as necessary, to the appropriate length in the trough (e.g., to 120 cm for 0-120 cm extraction and to 90 cm for the 120-210 cm extraction). Soil cores were divided into 15 cm increments. For each 15 cm depth increment the two soil cores taken per plot were combined into one composite sample.

In the 2015-2016 experiments, in the fall and spring, three to five 210 cm soil cores were taken per plot. Lancaster V had 5 in the fall and three in the spring;

Dorchester IB had four in the fall and three in the spring; Frederick IV had five in the fall (when possible, very rocky soil); Howard IB had three in the spring; Lancaster IB had three or four in the spring; Kent II had three in the spring. The cores were approximately equally spaced throughout the plot. Soil was extracted in three increments, from 0-60 cm deep, from 60-120 cm deep, and from 120-210 cm deep. Soil cores were emptied and arranged to the appropriate length in the trough. Soil cores were divided into 30 cm

increments. On Dorchester IB farm, soil cores were taken only to 180 cm, and the 120-180 soil core was kept as a 60 cm increment, rather than being divided into 30 cm increments. On Kent II farm, soil cores were taken only to 180 cm, and the 60-120 cm and 120-180 soil cores were kept as a 60 cm increment, rather than being divided into 30 cm increments. The multiple cores per plot were combined into one composite sample for each depth increment.

On six farms, corn plants and soil cores were sampled in June when corn reached the V5 (five leaf collar) growth stage and at least 30 cm tall (Table 20). Soil sampling was based on the procedures for pre-sidedress nitrate testing (PSNT) (Cornell University Cooperative Extension, 2012).

Soil cores were taken at eight points per plot (or subplot when applicable). Areas within the 1 m edge of the plot were avoided. Soil cores were placed randomly (not all in corn rows or between corn rows). Soil cores were taken from 0-30 cm deep. The soil cores were mixed and a composite sample was collected.

The closest corn plant to every soil sample was collected. Corn plant height measurements were taken from the soil to leaf height. When measuring the corn plant, the leaves were not pulled up; rather the height was the maximum height of the undisturbed plant. At the Kent II site, sections of corn rows were sampled rather than randomly located plants. Four meters of corn plants per plot were sampled (approximately 28 plants). A 1 m segment from the two center rows of corn in a plot, on both ends of the plot were sampled. The 1 m segments were 1 m in from edge of plot. Corn plant height was not recorded.

Corn plants were cut 1 cm from the soil. The corn was dried at 40 °C until it reached a constant weight. The corn was ground to 1 mm sieve size. For the Kent II farm, only 1/3 of the corn samples (about 9 plants) were ground. Biomass samples were dried in paper bags at 40 °C until a constant weight was attained. Dry biomass weights were recorded. The biomass was ground to 1 mm sieve size. Biomass samples were analyzed for total C and N at University of Maryland Department of Environmental Science and Technology Analytical Lab (LECO CN628 Elemental Analyzer LECO Corp., St. Joseph, MI).

In the field, the soil samples were put into sealed plastic bags and stored on ice for transport to the lab. The soil samples were dried at 40 °C for at least 48 hours, and the soil was sieved through a 2 mm sieve. The weight of the soil at the field moisture level, the weight of the soil after drying, and the weight of the gravel that did not pass through the 2 mm sieve was determined.

Exchangeable NO₃ and NH₄ in the soil was extracted with 0.5 *M* potassium sulfate (K₂SO₄) solution. Two grams of dry soil were mixed with 20.0 ml of 0.5 *M* K₂SO₄ in 50 ml tubes. The tubes were shaken at 200 rpm for 30 minutes and then settled undisturbed for 10 minutes. The liquid from the tubes was filtered through VWR 410 filter paper. The filtrate was tested for NO₃-N and NH₄-N using a Lachat QuikChem 8500 Automated Ion Analyzer (Hach Company, Loveland, CO). The filtrate was analyzed for NH₄-N by the salicylate method and for NO₂-N and NO₃-N by cadmium reduction method. The measured NO₃-N and NH₄-N (mg NO₃-N L⁻¹ or mg NH₄-N L⁻¹) was blank-corrected with filtered 0.5 *M* K₂SO₄ solution samples and converted to mg NO₃-N or NH₄-N kg soil⁻¹ (**Appendix 3**).

In order to convert values of NO₃-N and NH₄-N concentrations in the soil to stock amounts of NO₃-N and NH₄-N in kg ha⁻¹, soil bulk density values were estimated from dry mass of known soil volumes in the cores and corrected for gravel content (**Equation 18**). The mass and volume of soil was determined for each of the soil cores taken with the Veihmeyer probe. Bulk density values for each farm were based on the average of all cores from that farm for a given depth increment (e.g., 0-120 cm or 120-210 cm) (**Appendix 10**).

Equation 18 Bulk density of soil

$$\frac{g \, soil}{cm^3} = \, \frac{(g \, soil + gravel) - g \, gravel}{(\pi r^2 * height) - (\frac{g \, gravel}{2.65 \, g \, cm^{-3}})}$$

Where,

r = radius (in cm) of soil core, as determined by measuring the inside diameter of soil core tip to three significant figures and dividing by 2.

height = length (in cm) of the increment of soil collected estimated bulk density of gravel = 2.65 g cm^{-3}

Farm topsoil (0-30 cm) amounts of NO₃-N and NH₄-N (kg N ha⁻¹) and sand/clay/silt fractions was determined on late-summer samples, prior to establishing the cover crop treatments, with the exceptions of four farms: for Lancaster II and Huntington IA November control plot samples were analyzed, for Frederick III and Lancaster III April control plot samples were analyzed.

The pH was analyzed by a glass combination pH electrode and a pH meter (Metler Toledo InLab®413 combination meter). Soil particle size analysis was performed according to the modified pipette method (Gavlak, et al., 2005). Total C and N analysis

was performed at University of Maryland Department of Environmental Science and Technology Analytical Lab on LECO CN628 Elemental Analyzer (LECO Corp., St. Joseph, MI; Nelson and Sommers, 1996; Matejovic, 1993). Soil organic matter (SOM) (Loss on Ignition Method) and nutrient content by Mehlich3 extraction (P, K, Mg, Ca, Na, S) was measured at WayPoint Analytical, Inc (Richmond, VA).

Corn silage and grain yield

Corn grain or silage yield was estimated by taking hand-samples or using a harvester (Table 20). When corn silage or grain was estimated by taking hand-samples, the harvest area per plot was two rows by 6.10 m long. The harvest area was at least 1.52 m from plot ends and there were at least two border rows of corn between the edge of the plot and harvested row.

For corn silage harvest, corn plants were cut 4 cm from the ground and weighed using a hanging scale and tarp. From the harvested corn plants, a subsample of 6-11 plants was selected. For each subsample, the stover (including stalk and ear husk) and corn ears (grain + cob) were weighed separately, then dried at 40 °C and re-weighed. The wet and dry weights for each subsample were used to determine the whole plant percent moisture. The corn silage yield was estimated by dividing the whole plant dry weight by the harvest area (**Appendix 11**, Equation 19).

For the corn grain yield measurements, within the harvest area the corn ears were husked and broken off from the plant (leaving the husk attached to the stover). The ears were weighed. From the harvested ears, seven to nine ears were randomly selected as a subsample and weighed. The ears were brought back to the lab and dried at 40 °C until the weights remained constant. The dry weights were determined for corn ears. The wet

and dry weights for the corn ears were used to determine the ear percent moisture. The proportion of grain in the ear and the percent moisture of the ear were used to estimate the dry grain weight adjusted to 15.5% moisture. The corn grain yield was estimated by dividing the grain weight at 15.5% moisture by the harvest area (**Appendix 11**, Equation 20).

Statistical Analyses

All analyses were performed using SAS version 9.4 statistical software (SAS Institute, Cary, NC). The level of probability considered significant was p < 0.05, unless otherwise stated. Using Proc Mixed, an analysis of variance (ANOVA) was performed for fall and spring measurements of cover crop parameters (biomass, N content, C/N ratio) and soil parameters (amounts of NO₃-N and NH₄-N for 0-90 cm soil, 90-210 cm soil, and every 30 cm soil layer increment from 0 to 210 cm deep). Cover crop treatment was the fixed effect. Block or block within site was the random effect. Analyses were performed 1) separately for each site, 2) across sites for fall measurements (six sites for soil parameters, 13 sites for cover crop parameters), and 3) across sites for spring measurements (11 sites for soil parameters, 11 sites for cover crop parameters).

Using Proc Corr, a Pearson product-moment correlation was performed relating cover crop biomass (fall radish, fall winter cereal, fall + spring winter cereal) to weather factors (GDD, precipitation) and soil characteristics (topsoil NO₃-N and NH₄-N, topsoil percent sand, clay, and silt).

For each corn trial experiment site in which V5 corn and PSNT soil samples were taken, an ANOVA was performed investigating if the independent variable of cover crop treatment affected V5 corn biomass, V5 corn N content, and 0-30 cm soil NO₃-N and

NH₄-N concentrations. Across the six sites that had V5 corn and PSNT soil samples taken, these same variables were analyzed with rep(site) as a random factor in the analysis.

For each site at which corn yield samples were taken, an ANOVA was performed investigating if the independent variable of cover crop treatment, and in some cases the independent variable of fertilizer N rate and the interaction of cover crop treatment x fertilizer N rate, affected corn yield. Across the six sites that had corn grain samples taken in plots with the normal N fertilizer rate of the farm, and the six farms that had corn grain samples taken in the no fertilizer plots, an ANOVA was performed, with site(rep) as a random factor in the analysis, investigating if the independent variable of cover crop treatment affected the dependent variable of corn yield.

Results

Cover crop effects on soil inorganic N 0-210 cm deep

In late-fall, across six farms, for the 0-30 cm and 30-60 cm deep soil increments, the NO₃-N (kg ha⁻¹) was significantly higher in the control treatment than the radish, winter cereal, or mix treatments, and for the 60-90 cm soil increment, NO₃-N (kg ha⁻¹) was significantly higher in the control treatment than the radish treatment. In late-fall, for the 0-30 cm deep soil increment, the NH₄-N (kg ha⁻¹) averaged across six farms was significantly lower in the control treatment than the mix treatment (Table 21).

In the spring, across 11 farms, in every 30 cm soil increment from 0-210 cm, soil in the winter cereal treatment had significantly lower NO₃ than soil in the control treatment. In soil increments from 30-210 cm deep, soil in the mix treatment had

significantly lower NO₃ than soil in the control treatment, and the same level of NO₃ as soil in the winter cereal treatment; from 0-30 cm deep, soil in the mix treatment had the same level of NO₃ as soil in the control and radish treatments and higher NO₃ than soil in the winter cereal. From 0-30 cm deep, soil in the radish treatment had significantly higher NO₃ than soil in control or winter cereal. From 30-60 cm deep, soil in the radish treatment had significantly higher NO₃ than soil in winter cereal or mix treatments (and the same level as control). In each 30 cm increment from 60-150 cm deep, soil in the radish treatment had significantly higher NO₃ than soil in the winter cereal, the same level of NO₃ as soil in mix, and lower NO₃ than soil in control. In each 30 cm increment from 150-210 cm deep, soil in radish had significantly higher NO₃ than soil in control and the same level of NO₃ as soil in mix and winter cereal. Across 11 farms, the soil NH₄-N (kg ha⁻¹) did not differ at any soil depth increment (Table 21).

Site by site findings

The fall and spring soil NO₃-N and NH₄-N from 0-90 cm and 0-210 cm for each farm site is listed in Table 22. Figure 15 shows cover crop N uptake and the November residual soil NO₃-N levels for late-fall and/or spring samples.

Soil nitrate by depth increment

Dorchester IB—In the fall, there were no differences among cover crop treatments for soil NO₃-N in any 30 cm layer. In the spring, control had more NO₃-N than mix in soil layers from 30-120 cm, control had more NO₃-N than winter cereal in soil layers from 30-90 cm, and control had more NO₃-N than radish in soil layers from 60-120 cm (Figure 16). **Frederick I**—In the fall, control had more NO₃-N than mix in soil layers from 60-120 cm. (No fall soil samples were taken in radish or winter cereal.) In the

spring, control had more NO₃-N than radish, winter cereal, and mix in 30-60 cm soil, and control had more NO₃-N than winter cereal and mix in the 60-90 cm soil (Figure 16). Frederick III—In the spring, control had more NO₃-N than radish, mix, or winter cereal in soil layers from 30-60 cm deep (Figure 16). Frederick IV—In the fall, control had more NO₃-N than radish or winter cereal in soil layers from 0-90 cm (Figure 16). **Harford I**—In the fall, control had more NO₃-N than radish or mix in soil layers from 0-60 cm, and radish had more NO₃-N than mix and control in 180-210 cm soil (Figure 16). **Howard IA**— In the fall, there were no differences among cover crop treatments for soil NO₃-N in any 30 cm layer (although only two replications were sampled). No soil samples were taken in the spring. **Howard IB**—In the spring, radish had more NO₃-N than mix, control, and winter cereal, and control and mix had more NO₃-N than the winter cereal in the 0-30 cm soil. Radish had more NO₃-N than mix, control, and winter cereal in the 30-60 cm soil. Control and radish had more NO₃-N than winter cereal, and control had more NO₃-N than mix in 60-90 cm soil. Control had more NO₃-N than winter cereal in 90-120 cm soil (Figure 16). **Huntington IA**—In the fall, control had more NO₃-N than radish, winter cereal, and mix in 0-30 cm soil. In the spring, mix had more NO₃-N than control, and mix, radish, and control had more NO₃-N than winter cereal in 0-30 cm soil. Mix and control had more NO₃-N than winter cereal in 30-60 cm soil. Control had more NO₃-N than winter cereal in soil layers from 0-90 cm and 120-180 cm (Figure 16). **Kent II**— In the spring, there were no differences among cover crop treatments for soil NO₃-N in any 30 cm layer. No soil samples were taken in the fall. **Lancaster IA**—In the fall, control had more NO₃-N than winter cereal in soil layers from 30-90 cm deep. Control had more NO₃-N than radish and mix in 30-60 cm soil. In the spring, control had

more NO₃-N than winter cereal, radish, and mix in the soil layers from 60-150 cm and 180-210 soil (Figure 16). Lancaster IB—In the spring, radish and control had more NO₃-N than winter cereal, mix and late-mix in 30-60 cm soil. Control had more NO₃-N than radish, winter cereal, mix and late-mix in soil layers from 60-120 cm (Figure 16). Lancaster II—In the fall, control had more NO₃-N than winter cereal and radish in soil layers from 0-60 cm, and control had more NO₃-N than mix in 0-30 cm soil. In the spring, control had more NO₃-N than winter cereal in soil layers from 30-120 cm, control had more NO₃-N than mix in soil layers from 30-90 cm deep, and control had more NO₃-N than radish in 60-90 cm soil (Figure 16). Lancaster III—In the spring, radish had more NO₃-N than mix in soil layers from 0-60 cm. radish and control had more NO₃-N than winter cereal and mix in soil layers from 90-150 cm. Radish had more NO₃-N than mix in 150-180 cm soil (Figure 16). Lancaster V—In the fall, control had more NO₃-N than winter cereal, mix, and radish in soil layers from 0-120 cm deep. In the spring, radish had more NO₃-N than winter cereal and control in soil layers from 0-60 cm. Control had more NO₃-N than radish and winter cereal from 60-90 cm. control had more NO₃-N than winter cereal in soil layers from 90-120 cm. Radish had more NO₃-N than winter cereal in 120-150 cm soil. Radish and control had more NO₃-N than winter cereal in 150-180 cm soil (Figure 16). (No spring soil samples were taken in mix.)

Soil ammonium by depth increment

Lancaster IA, Lancaster II, and Lancaster V were the only farms out of the 14 to have significant differences in NH₄-N at any depth increment. For **Lancaster IA**, in the fall, there were no differences between cover crop treatments for NH₄-N levels. In the spring 0-180 cm soil, the control treatment had higher NH₄-N than the triticale treatment.

For **Lancaster II**, in the fall, in the soil layers from 0-120 cm, the mixed species cover crop treatment had significantly more NH₄-N than the control and radish treatments, and from 30-60 cm and 90-120 cm more than the triticale treatment. From 180-210 cm deep, the triticale treatment had significantly more NH₄-N than the radish and control treatments. In the spring, there were no differences between cover crop treatments for NH₄-N levels. For **Lancaster V**, in the fall, in soil layers 0-30 cm and 90-120 cm, the mixed species cover crop treatment had significantly more NH₄ than the control and radish treatments. In the spring, there were no differences between cover crop treatments for NH₄ levels.

Dorchester IB, Frederick I, and Huntington IA, farms had no differences between cover crop treatments at any soil depth for NH₄-N amounts in the fall or spring. Frederick IV, Harford I, and Howard IA farms had no differences between cover crop treatments at any soil depth for NH₄-N amounts in the fall and samples were not taken in the spring. Frederick III, Howard IB, Kent II, Lancaster IB, and Lancaster III farms had no differences between cover crop treatments at any soil depth for NH₄-N amounts in the spring and samples were not taken in the fall.

Cover crop growth and N content

The fall and spring cover crop biomass and cover crop N content for each farm site is indicated in Table 22. In the fall, across the 13 farms, radish biomass was significantly higher than mix, which was significantly higher than winter cereal. The N content was significantly higher for radish and mix than for winter cereal. The C/N ratio did not differ between radish, mix and winter cereal cover crop treatments (**Table 23**).

In the spring, across the 10 farms, winter cereal biomass was higher than mix. There were no significant differences between the N uptake of winter cereal and mix. The C/N ratio was greater for winter cereal than mix (**Table 23**).

When comparing radish to winter cereal prior to their termination (i.e., naturally winter-killing for radish or oat (*Avena sativa* L.) and chemically terminated with herbicide for rye (*Secale cereale* L.) and triticale (x *Triticosecale* Wittm. ex A. Camus) winter cereals, biomass was not significantly different between radish and winter cereal (p = 0.9251). The N content was significantly higher for radish (80.1 kg ha⁻¹) than winter cereal (58.7 kg ha⁻¹) at a significance level of p = 0.0013. The C/N ratio was significantly lower for radish (14.2) than winter cereal (22.6) at a significance level of p < 0.0001 (**Table 23**).

Relationships between cover crop biomass and environmental characteristics

The number of GDD was positively correlated to fall radish biomass (r = 0.43; p = 0.094) and spring winter cereal biomass (r = 0.63, p = 0.012). Precipitation was positively correlated to spring winter cereal biomass (r = 0.58; p = 0.025). Topsoil (0-30 cm) NO₃-N was positively correlated to fall winter cereal biomass (r = 0.77; p = 0.001) and to fall radish biomass (r = 0.48; p = 0.080). The percent sand in the topsoil was negatively correlated to fall winter cereal biomass (r = -0.52; p = 0.056), fall radish biomass (r = -0.47; p = 0.092), and spring winter cereal biomass (r = -0.74; p = 0.002). Topsoil nitrate was negatively correlated with topsoil percent sand in fall radish plots (r = -0.50; p = 0.070), fall winter cereal plots (r = -0.53; p = 0.050), and spring winter cereal plots (r = -0.57; p = 0.026). The topsoil percent silt was positively correlated to fall

winter cereal biomass (r = 0.52; p = 0.0582), fall radish biomass (r = 0.56; p = 0.0381), and spring winter cereal biomass (r = 0.70; p = 0.004) (Table 24).

Cover crop effect on PSNT soil and corn plants

Across the six farms on which PSNT soil samples (30 cm deep at corn V5 stage) were taken, the soil NO₃-N concentration for radish was significantly higher than for winter cereal and mix. The soil NH₄-N concentrations did not differ among any of the cover crop treatments. At the Howard IB site, radish NO₃-N concentration was higher than winter cereal, and at the Kent II site radish NO₃-N concentration was higher than mix and late-mix. Across the six farms, the V5 corn biomass plant⁻¹ and shoot N plant⁻¹ were significantly affected by the previous cover crop treatment in the order radish > mix = control > winter cereal (Table 25; Figure 18).

Cover crop effect on corn yield and corn response to N fertilizer

Averaged across six farms at the farmers' standard N fertilizer application rate, corn yield following radish or control was higher than winter cereal. Corn yield following radish was higher than mix. Averaged across six farms at the 0 fertilizer N rate, corn yield following radish > control = mix > winter cereal (Table 26).

For Franklin IIB (0 fertilizer N rate) and for Frederick IV (farmer's standard fertilizer N rate), there was no significant cover crop treatment effect on corn silage yield. For Howard IA, there was a corn grain yield response to cover crop treatment and N fertilizer treatment, but no interaction. Corn in radish and control yielded more than corn in winter cereal. Corn yield responded to fertilizer N in order of 0 kg ha⁻¹ < 67 kg ha⁻¹ < 157 kg ha⁻¹ = 202 kg ha⁻¹ (Figure 19). For Howard IB, there was a corn grain yield response to cover crop treatment, N fertilizer treatment, and the interaction between cover

crop x N fertilizer (p = 0.0889). At all fertilizer rates, corn in radish yielded higher than corn in winter cereal, and at some fertilizer rates corn in radish yielded higher than corn in mix or control. At 67 kg ha⁻¹, 135 kg ha⁻¹, and 202 kg ha⁻¹ fertilizer rates, corn in mix yielded higher than corn in winter cereal. Corn yields following radish and control did not increase between N application levels of 135 and 202 kg ha⁻¹ fertilizer N. Corn yields following winter cereal and mix (p = 0.055) increased with 202 kg ha⁻¹ fertilizer N in comparison to 135 kg ha⁻¹ fertilizer N (Figure 19). For Frederick I, there was a corn grain yield response to cover crop treatment and N fertilizer treatment, but no interaction. Corn in radish and control yielded more than corn in winter cereal, and corn in radish yielded more than corn in mix. Corn yield responded to fertilizer N in order of 0 kg ha⁻¹ < 112 kg ha⁻¹ = 168 kg ha⁻¹ (Figure 19). For Lancaster IA, there was a corn grain yield response to the interaction between cover crop x N fertilizer. In the 0 kg ha⁻¹ fertilizer N treatment, corn in winter cereal yielded less than corn in radish, mix, or control. Also, corn yield responded to fertilizer N in order of 0 kg ha⁻¹ < 140 kg ha⁻¹ = 168 kg ha⁻¹ = 224 kg ha⁻¹ in winter cereal. Corn in radish, mix, and control did not respond to fertilizer N (Figure 19). For Lancaster IB, there was a corn grain yield response to cover crop treatment and N fertilizer treatment, but no interaction. Corn in radish and control yielded more than corn in winter cereal, and corn in radish yielded more than corn in early-planted mix. Corn with 135 kg N ha⁻¹ yielded more than corn with 67 kg N ha⁻¹ (Figure 19).

Discussion

Cover crop effects on soil inorganic nitrogen 0-210 cm deep

Radish, winter cereal, and mix cover crops each performed differently in terms of reducing deep soil NO₃ in the fall and spring and increasing surface soil NO₃ in the spring. Radish was the most effective cover crop at reducing the soil NO₃ in the fall from the deeper soil layers and ensuring available NO₃ on the soil surface (0-30 cm) in the spring. In the fall, radish reduced NO₃ pools to 90 cm deep, while winter cereal or mix cover crops reduced NO₃ to only 60 cm deep. Other studies have also found radish to be more effective than rye at reducing levels of soil NO₃-N by late-fall, especially in deep layers (> 1 m) (Thorup-Kristensen, 2001; Thorup-Kristensen, et al., 2009; Kristensen and Thorup-Kristensen, 2004a).

However, in the spring, radish was less effective than winter cereal at reducing soil NO₃ from 30-150 cm deep. Winter cereal was the most effective at reducing soil NO₃ in the spring throughout the entire soil profile. Mix was more effective than winter cereal and as effective as radish at ensuring available NO₃ on the soil surface (0-30 cm) in the spring, and was as effective as winter cereal in reducing soil NO₃ from 30-210 cm soil. Several other studies found that in the spring, rye or radish cover crops did not reduce upper layer (0-0.5 or 1 m) soil NO₃-N content compared to the no cover crop control, but they did reduce deep soil (> 1 m) soil NO₃-N content (Thorup-Kristensen, 2006b; Thorup-Kristensen, et al., 2009).

At most sites in the current study, soil NH₄-N did not differ between cover crop treatments. Other studies have found that NH₄-N levels in the soil tend to be less variable among cover crop or crop species than NO₃ levels (Kristensen and Thorup-Kristensen, 2004a; Kristensen and Thorup-Kristensen, 2004b). For example, Bergstrom (1986) took soil cores 1 m deep six to nine times per year in cropping systems of barley (*Hordeum*

vulgare L.), barley with 120 kg N ha⁻¹ fertilizer, fescue (*Festuca* L.) with 200 kg N ha⁻¹ fertilizer, and alfalfa (*Medicago sativa* L.) with no fertilizer. Ammonium amounts did not vary much between treatments, staying between 11 and 13 kg N ha⁻¹, whereas NO₃-N ranged between 23 and 68 kg N ha⁻¹ (Bergstrom, 1986). On some sites in the current study, NH₄-N was higher in cover crop treatments than control no cover crop. Lacey and Armstrong (2015) found that the NH₄-N of radish and rye cover crops was higher than no cover crop in the spring of one of two study years, while the other year there were no differences, possibly attributable to cold and wet soil conditions slowing mineralization rates.

Cover crop growth and N content

In terms of fall growth, radish was more productive than winter cereal or mix cover crops. Kristensen and Thorup-Kristensen (2004a) and Thorup-Kristensen (2001) also found radish had higher biomass and N accumulation than rye. We found that the radish biomass was weakly correlated with GDD, while fall winter cereal biomass was not correlated to GDD. Radish is more sensitive to cold weather than winter cereal cover crops (Lacey and Armstrong, 2015; Thorup-Kristensen, et al., 2003). As expected, spring winter cereal biomass was correlated to GDD. Both radish and winter cereal biomass increased with lower percent sand and more NO₃ in the topsoil, which was expected as NO₃ would more readily leach from sandy soils and become less available to support cover crop growth.

Radish winter-killed at all farm sites. For samples taken shortly before winter-kill, the average C/N ratio was 14.2. When C/N ratios are < 25/1, N will generally be plant available and not immobilized (Weil and Brady, 2017). Therefore, N released from the

dead radish biomass was likely available for plant uptake and not immobilized. The cover crop biomass shortly before termination for radish and winter cereal did not differ, but on average radish had accumulated 21.4 kg ha⁻¹ more biomass N than winter cereal, and radish biomass had a C/N ratio of 14.2 while the winter cereal C/N ratio was 22.6. Therefore, the radish cover crop had both greater N accumulation in the biomass and a lower likelihood of N immobilization due to the lower C/N ratio. Winter cereal cover crops commonly have C/N ratios higher than mixes. On a loamy sand soil in North Carolina, the C/N ratio for October-planted cover crops in April was greatest to smallest in the order of rye monoculture (38) > rye/clover mix (27) > crimson clover monoculture (16) (Ranells and Wagger, 1997). For a hairy vetch/rye mix cover crop, as vetch went from comprising 0 to 100% of the mixture, the N content increased (64 to 181 kg N ha⁻¹) and C/N ratio decreased (83 to 16) (Poffenbarger, et al., 2015b).

Corn yield had a negative linear relationship with cover crop C/N ratio ($R^2 = 0.55$) and a positive relationship with cover crop biomass ($R^2 = 0.23$) (Finney, et al., 2016). White, et al. (2016) found that to maximize cover crop N supply and provide the greatest yield benefit to a subsequent corn crop, cover crops should have low C/N ratio and high biomass N content. Species that fit these criteria, based on experiments performed in Pennsylvania, include legumes such as fava bean (*Vicia faba* L.), red clover (*Trifolium pretense* L.), and hairy vetch (*Vicia villosa* Roth.), grown in monoculture or in mixtures with each other or with grasses including triticale, Italian ryegrass, or oat or a brassica forage radish. Thomsen, et al. (2016) found during incubation studies with forage radish, white mustard (*Sinapis alba* L.), and perennial ryegrass using a loamy sand soil, that the residue C/N ratio and N concentration were the best single predictors for net

N mineralization, regardless of temperature, or of cover crop type, age, or planting date. It is important to note that the timing of the mineralization is also important, since it is possible that N could mineralize and leach out of the rooting zone before spring crops take it up.

Corn growth and yield

In June, at PSNT sampling, June soil NO₃-N concentrations were positively related to the fraction of total corn yield. Corn with a preceding rye cover crop tended to have lower soil NO₃-N concentrations and corn yields, while corn with a preceding radish cover crop tended to have higher soil NO₃-N and corn yields (Figure 17). The critical value for PSNT NO₃-N concentrations is 21 mg N kg⁻¹ soil. If the NO₃-N concentration is greater than 21 mg N kg⁻¹ soil, sidedress N fertilizer is not recommended in Maryland (University of Maryland Extension, 2010). The corn following all of the cover crop treatments fell below this threshold and required N sidedress application, but the radish treatment had higher topsoil NO₃-N than either the cereal or cover crop mixtures.

Nitrogen fertilizer response curves can help determine if more or less fertilizer is required for optimal corn yields. At Howard IB farm, the N response plateaued for corn following radish cover crop, mix cover crop, or control cover crop at 135 kg ha⁻¹ N applications (farmer's normal rate), but the N response continued to increase for corn following rye cover crop even at 202 kg ha⁻¹ (150% of farmer's normal rate). The Lancaster IA farm had outstanding soil fertility and corn was minimally responsive to N fertilization in any of the treatments. The Howard IA corn showed minimal responses to N as well. On Frederick I farm, there was not a significant interaction between corn yield and fertilizer rate, although corn following radish and control had yields around 10 Mg

ha⁻¹ that leveled off at 112 kg ha⁻¹ N fertilizer application (100% farmer's normal rate), while corn following winter cereal had maximum yields of 7.6 Mg ha⁻¹ at 168 kg ha⁻¹ N fertilizer application (150% of farmer's normal rate).

While corn N fertilizer response is variable based on site fertility, in our study, corn following winter cereal tended to have lower yield and/or higher fertilizer requirements than corn following no cover crop or a radish or mixed cover crop. A metaanalysis including 47 studies with grass cover crops and 13 studies with mixed species cover crops found that corn following a mixed cover crop had 13% higher average yields than no cover crop, and mixed cover crops with late termination dates (0-6 days before corn planting) had 30% higher corn yield compared to no cover crop (Marcillo and Miguez, 2017). Corn following a grass cover crop was not different than no cover crop, however management practices such as the corn N fertilizer rate was a highly significant moderator of yield response (Marcillo and Miguez, 2017). Nitrogen in winter cereal cover crops is released very slowly by decomposition and is often immobilized by microbes utilizing the abundant carbon in the residues and is therefore largely unavailable for crop uptake (Adeli, et al., 2011; Doran and Smith, 1991; Ketterings, et al., 2015; Thorup-Kristensen and Dresbøll, 2010). As a result, higher levels of spring N fertilizer are often applied following winter cereal cover crops than would be applied without a cover crop.

We found that in some cases, radish improved corn yield in comparison to the no cover crop control. On a Hadley fine sandy loam soil in Massachusetts, Jahanzad, et al. (2017) found that potato (*Solanum tuberosum* L.) yield and yield components were higher for potato following forage radish cover crop than cereal rye or no cover crop.

Potato grown following forage radish produced the highest yield when fertilized with 75 or 150 kg N ha⁻¹, while potato grown following no cover crop produced the highest yield when fertilized with 225 kg N ha⁻¹ (Jahanzad, et al., 2017). In contrast, studies performed in Minnesota, Wisconsin, and Missouri indicated no fertilizer replacement value or benefit on corn yield of forage radish, despite substantial N uptake by the radish (Gieske, et al., 2016; Sandler, et al., 2015; Ruark, et al., 2018).

Conclusions and practical applications

Through performing on-farm trials of early-planted cover crop systems, we were able to observe a range of cover crop responses. Cover crops are affected by soil type, management, and weather. Furthermore, site-by-site results of residual soil NO₃-N and NH₄-N were often highly variable. Soil sampling can be challenging due to the heterogeneity of soil, more so in deep soil layers. Even with the variability among sites and soil samples, there were clear trends showing that early-planted forage radish and rye cover crops (monoculture or mix) can scavenge soil N from 1+ m. This is expected to reduce NO₃-N leaching.

Overall it can be concluded that winter cereal had a negative impact on the following corn. Either extra fertilizer will need to be added, which is contrary to the goal of improving the overall cropping system's nutrient use efficiency, or farmers' yields will be reduced, which is contrary to the goal of "making cover crops pay". On the other hand, a mixed species cover crop has no negative (or positive) impact on the cropping system, and a radish cover crop has a neutral or sometimes positive impact on the cropping system, in terms of improving the overall nutrient use efficiency. Cover cropping is a practice that is already widely adopted in Maryland, and planting cover

crops earlier in the fall can greatly increase their ability to scavenge and potentially release deep soil N.

Table 18. Site histories of cover crop studies.

Site	Year	Most recent crop	Crop rotation history	Manure history	Tillage history
Dorchester IB	2015-2016	wheat	NA ¹	NA	NA
Franklin I	2014-2015	corn	Corn, small gran silage, alfalfa	Regular	No-till
Franklin IIA	2014-2015	corn	Corn, small grain silage	Regular	Mostly no-till; Occasional tillage
Franklin IIB	2015-2016	corn	Corn, small grain silage	Regular	Mostly no-till; Occasional tillage
Frederick I	2014-2015	corn	NA	Regular	NA
Frederick III	2014-2015	NA	NA	Regular	NA
Frederick IV	2015-2016	corn	Double crop corn/triticale for > 5 years	Regular manure applications spring and fall for past 10 years	Subsoiled in 2014, disked 1x per year until 2016
Harford I	2014-2015	corn	NA	Regular	NA
Howard IA	2014-2015	corn	2014 corn silage, 2013 corn, 2012 forage sorghum, 2011 sweet corn, 2010 sweet corn	Occasional	No-till
Howard IB	2015-2016	corn	2015 corn silage, 2014 soybean, 2013 corn grain (rye cover crop), 2012 corn grain, 2011 corn grain, 2010 corn grain	No manure applications past 20+ years	No-till
Huntington IA	2014-2015	corn	Continuous corn with rye cover crop in winter	Regular	No-till
Huntington IB	2015-2016	corn	Continuous corn with rye cover crop in winter	Regular	No-till
Kent II	2015-2016	corn	NA	NA	NA
Lancaster IA	2014-2015	wheat	2013 pumpkin, 2012 corn, 2011 corn, 2010 soybean	Occasional	No-till past 5+ years
Lancaster IB	2015-2016	wheat	2014 pumpkin, 2013 corn, 2012 soybean, 2011 corn	Occasional	No-till past 5+ years
Lancaster II	2014-2015	tobacco	Silage corn, forage rye cover crop, tobacco, possibly alfalfa	Regular (every year except when growing tobacco)	Tobacco plowed and cultivated several times; corn maybe no-till

Lancaster III	2014-2015	corn	Silage corn, mostly grass cover crop, grain corn, alfalfa	Regular (every year except a half rate on top of alfalfa applied when dormant in fall or winter)	No-till			
Lancaster V	2015-2016	tobacco	Corn silage, forage rye, tobacco, alfalfa rotation	Regular	Mostly no-till corn, some no-till tobacco			
Prince Georges I	2014-2015	corn	2013 soybean, 2012 corn, 2009-2011 mixed grass hay with < 25% legumes	No manure ever applied	No-till past 5+ years			
¹ Data not available indicated as NA								

Table 19. Cover crop treatments, planting date, management details, and sampling timing of cover crop studies.

Site	Plot size	Cover crop treatments with seeding rates, kg ha ⁻¹	Cover crop application	Date planted	Cover crop samples Soil samples						nples	
						Fall		Spring			Fall	Spring
					Date	GDD	Prec., mm	Date	GDD	Prec., mm	Date	Date
Dorchester IB	69 m x 6.9 m	radish; wheat; mix (radish + annual ryegrass + Crimson clover); control	drilled	23 Aug 2015	4 Dec (rep 1); 5 Dec (rep 2); 6 Dec 2015 (rep 3)	2334; 2341; 2349	287	17 Apr 2016	3560	709	4 Dec 2015	28 May 2016
Franklin I	121 m x 9.1 m	triticale @ 90; radish @ 6.7; mix (triticale @ 72 + radish @ 6 + crimson clover @ 12); control	drilled	15 Sep 2014	21 Nov 2014	978	153	1 Apr 2015	1257	414	none	none
Franklin IIA	15 m x 4.6 m	oats @ 112; radish @ 6.7; mix (oats @ 90 + radish @ 5.6); control	drilled	10 Sep 2014	21 Nov 2014	1179	114	winter kill	N/A	N/A	none	none
Franklin IIB	61 m x 4.6 m	oats @ 112; radish @ 6.7; mix (oats @ 90 + radish @ 5.6); control	drilled	30 Aug 2015	14 Nov 2015	1706	145	winter kill	N/A	N/A	none	none
Frederick I	49 m x 4.6 m	radish @ 8.8; triticale @ 135; mix (triticale @ 67 + radish @ 4 + crimson clover @ 10); control	drilled	5 Sep 2014	1 Dec 2014	1295	193	9 Apr 2015	1669	522	12/1/2014	4/13/2015
Frederick III	55 m x	radish @ 21.4; triticale @ 135; mix	drilled	15 Sep 2014	none	none	none	9 Apr 2015	1387	515	none	4/23/2015

	4.6 m	(triticale @ 67 + radish @ 7.7 + crimson clover @ 19); control										
Frederick IV	61 m x 4.6 m	radish @ 5.6; triticale @ 146; control	drilled	4 Sept 2015	7 Nov (rep 1- 3); 14 Nov 2015 (rep 4)	1317; 1389	249; 274	24 Apr 2016	2527	718	7 Nov (rep 1-3); 14 Nov 2015 (rep 4)	none
Harford I	91 m x 9.1 m	radish; mix (radish + annual ryegrass + crimson clover); control	broadcast, lightly covered	4 Sep 2014	16 Nov 2014	1310	168	none	N/A	N/A	16 Nov 2014	none
Howard IA	61 m x 3.0 m	rye @ 129; radish @ 8.7; control	drilled	15 Sep 2014	9 Nov 2014	972	142	16 Apr 2015	1566	632	9 Nov 2014	none
Howard IB	61 m x 3.0 m	radish @ 7.5; rye @ 139; mix (triticale @ 40, radish @ 3.4, clover @ 6.7); control	drilled	1 Sep 2015	6 Jan 2016	2034	410	24 Apr 2016	2841	777	none	24 May 2016
Huntington IA	61 m x 9.1 m	radish @ 9.0; rye @ 126; mix (oat @ 72 + radish @ 5.6); control	drilled	10 Sep 2014	15 Nov 2014	935	134	25 Apr 2015	1338	542	15 Nov 2014	25 Apr 2015
Huntington IB	61 m x 9.1 m	radish @ 11; ryegrass @ 20; mix (ryegrass @ 5.07 + radish @ 1.30 + crimson clover @ 12.6 + red clover @ 1.95 + sweet clover @ 0.39); control	drilled	10 Sep 2015	6 Dec 2015	1223	222	none	N/A	N/A	none	none

Kent II	152 m x 14 m	radish; rye; mix (radish + rye + crimson clover); control	drilled	11 Sep 2015; late-mix 24 Sep 2015	5 Dec 2015	1588	276	17 Apr 2016	2600	569	none	25 May 2016
Lancaster IA	15 m x 3.0 m	radish @ 3.4; triticale @ 45; mix (ryegrass @ 10 + radish @ 2.2 + clover @ 4.5); control	drilled	18 Aug 2014	19 Dec 2014	2074	304	28 Apr 2015	2593	583	19 Dec 2014	7 May 2015
Lancaster IB	15 m x 3.0 m	radish @ 3.4; triticale @ 45; mix (triticale @ 40 + radish @ 3.4 + clover @ 6.7); control	drilled	4 Sep 2015; late-mix 30 Sep 2015	28 Nov 2015	1604	284	24 May 2016	3075	820	none	24 May 2016
Lancaster II	31 m x 4.6 m	radish @ 9.0; triticale @ 135; mix (triticale @ 67 + ryegrass @ 22 + radish @ 5.6 + clover @ 17); control	drilled	4 Sep 2014	12 Nov 2014	1282	176	11 Apr 2015	1694	574	12 Nov 2014	13 Apr 2015
Lancaster III	31 m x 4.6 m	radish @ 9.0; triticale @ 135; mix (triticale @ 67 + ryegrass @ 22 + radish @ 5.6 + clover @ 17); control	drilled	11 Sep 2014	none	N/A	N/A	27 Apr 2015	1617	630	none	27 Apr 2015
Lancaster V	15 m x 6.5 ft	radish @ 9.0; triticale @ 123; mix (triticale @ 56 + ryegrass @ 22 + clover @ 17 + radish @ 1.1); control	drilled	2 Sep 2015	6 Nov 2015 (rep 1), 13 Nov 2015 (rep 2- 4)	1496; 1577	225; 239	16 Apr 2016	2609	682	6 Nov (rep 1); 13 Nov (rep 2), 24 Nov (rep 3); 28 Nov rep 4	16 Apr 2016

Prince Georges I	31 m x 6.1 m	radish @ 13; rye @ 101, wheat @ 101; mix (ryegrass @ 11 + radish @ 2.5 + clover	drilled	4 Oct 2014	14 Nov 2014	661	108	20 Apr 2015	1454	592	none	none
		@ 5.1); control										

Table 20. Corn yield and N response trials. Cover crop termination date, corn planting date, N fertilizer type, date applied and rates, corn herbicide information, samples taken and sampling method.

Site	Cover crops terminat ed	Corn planti ng date	N fertilizer type	N rates	Date N applied	Herbicide applied	V5 corn & PSNT soil sampl	Corn harvest method	Corn harve st date
Dorchester IB (2016)	6 Jun 2016 ¹	8 Jun 2016	NA ²	NA	NA	NA	yes	Crop failure ³	
Franklin IIB (2015)	All winter killed	5 May 2016	NA	0 kg ha ⁻¹	NA	Unknown	yes	Silage (hand harvest)	19 Aug 2016
Frederick I (2015)	NA	NA	30% UAN	0 kg ha ⁻¹ , 56 kg ha ⁻¹ , 112 kg ha ⁻¹ , 168 kg ha ⁻¹	16 May 2015	2-chloro-4-ethylamino-6-isopropylamino-s-triazine @ 0.56 kg active ing. ha ⁻¹ ; S-metolachor @ 0.975 kg active ing. ha ⁻¹ ; mesotrione @ 0.126 kg active ing. ha ⁻¹ ; atrazine @ 0.975 kg active ing. ha ⁻¹ ; Simazine: 2-chloro-4,6-bis(ethylamino)-s-triazine @ 0.560 kg active ing. ha ⁻¹ ; Dimethylamine salt of 2,4-Dichlorophenoxyacetic acid @ 0.532 kg active ing. ha ⁻¹ ; glyphosate, N-(phosphonomethyl)glycine @ 2.31 kg active ing. ha ⁻¹	no	Grain (harvest er)	12 Oct 2015
Frederick IV (2016)	25 Apr 2016	25 Apr 2016	NA	NA	NA	NA	yes	Silage (hand harvest)	26 Aug 2016
Howard IA (2014)	18 Apr 2015	9 May 2015	30% UAN	0 kg ha ⁻¹ 67 kg ha ⁻¹ 157 kg ha ⁻¹ 208 kg ha ⁻¹	12 Jun 2015; sidedress	9 May 2015; atrazine @ 1.74 kg active ing. ha ⁻¹ ; S-metolachor @ 1.35 kg active ing. ha ⁻¹ ; Paraquat @ 0.468 kg active ing. ha ⁻¹	no	Grain (harvest er)	29 Sep 2015
Howard IB (2015)	18 May 2016	30 May 2016	30% UAN	0 kg ha ⁻¹ 67 kg ha ⁻¹ 135 kg ha ⁻¹	27 Jun 2016; sidedress	18 May 2016; atrazine @ 1.74 kg active ing. ha ⁻¹ ; S-metolachor @ 1.35 kg active ing. ha ⁻¹ ; Paraquat @ 0.468 kg active ing. ha ⁻¹	yes	Grain (harvest er)	18 Oct 2016

				202 kg ha ⁻¹					
Kent II (2015)	At corn planting	13 May 2016	32% N UAN	0 kg ha ⁻¹ 87 kg ha ⁻¹ 174 kg ha ⁻¹ 261 kg ha ⁻¹	10 Jun 2016	Chemicals at planting; S- Metolachlor; Atrazine; Mesotrione; Simazine; Paraquat; Lambda-cyhalothrin (amounts NA)	yes	None	NA
Lancaster IA (2015)	15 May 2015	29 Apr 2015	50/50 blend of Super Urea and Ammoni um Sulfate	0 kg ha ⁻¹ 84 kg ha ⁻¹ 140 kg ha ⁻¹ 168 ⁴ kg ha ⁻¹ 224 kg ha ⁻¹	4 Jun 2015; sidedress	S- Metolachlor @ 1.80 kg active ing. ha ⁻¹ ; Atrazine @ 0.673 kg active ing. ha ⁻¹ ; Mesotrione @ 0.180 kg active ing. ha ⁻¹	no	Grain (harvest er)	23 Sep 2015
Lancaster IB (2016)	28 May 2016	25 May 2016	28% UAN at planting; 50/50 blend Super Urea and Ammoni um sulfate sidedress	67 kg ha ⁻¹ at planting; 135 ⁴ kg ha ⁻¹	23 Jun 2016	S- Metolachlor @ 1.80 kg active ing. ha ⁻¹ ; Atrazine @ 0.673 kg active ing. ha ⁻¹ ; Mesotrione @ 0.180 kg active ing. ha ⁻¹	yes	Grain (hand harvest)	23 Sep 2016

¹ Sprayed field with *S*-metolachor, mesotrione, atrazine (Lexar) and glyphosate, N-(phosphonomethyl)glycine (RoundUp)

² Data not available indicated as NA

³ Crop failure due to deer damage

⁴ Split between planting and sidedress time

Table 21. Soil NO₃-N and NH₄-N (kg ha⁻¹) of radish, winter cereal (cereal), mixed species (mix), and control cover crop treatments for six farms for late-fall sampling and for 11 farms for spring sampling. Different letters indicate statistically significant differences between cover crop treatments per depth increment. Farms sampled in late-fall include Dorchester IB, Frederick IV, Huntington IA, Lancaster II, and Lancaster V. Dorchester IB cores only to 180 cm deep, Frederick IV did not have soil core samples from mix treatment. Farms sampled in spring include Dorchester IB, Frederick II, Howard IB, Huntington IA, Lancaster IA, Lancaster II, Lancaster III, Lancaster V, and Kent II. Dorchester IB and Kent II soil cores were to only 180 cm deep. Lancaster V did not have soil core samples from mix treatment.

Depth increment	Radish	Cereal	Mix	Control	Radish	Cereal	Mix	Control
cm		- Soil NO ₃ -	N (kg ha ⁻¹)			Soil NH ₄ -N	(kg ha ⁻¹)	
			Late-f	all sampling	Ţ			
0-30	21.8 a	22.5 a	26.8 a	69.7 b	28.6 ab	31.1 ab	36.8 a	23.0 b
30-60	13.4 a	14.1 a	22.5 a	41.6 b	14.6 a	16.9 a	18.9 a	12.7 a
60-90	13.7 a	18.0 ab	20.0 ab	29.0 b	13.1 a	15.0 a	12.0 a	10.9 a
90-120	16.2 a	19.5 a	23.8 a	24.0 a	11.9 a	14.9 a	17.6 a	11.0 a
120-150	18.8 a	22.8 a	22.4 a	25.9 a	15.0 a	21.5 a	19.8 a	15.9 a
150-180	22.1 a	20.5 a	23.7 a	23.3 a	16.7 a	19.0 a	20.9 a	17.4 a
180-210	26.8 a	21.9 a	24.3 a	25.8 a	15.0 a	19.7 a	18.7 a	15.6 a
30-90	27.1 a	32.0 a	42.5 ab	70.6 b	27.6 a	31.8 a	31.0 a	23.6 a
90-150	35.0 a	42.3 a	46.1 a	50.3 a	27.0 a	36.3 a	37.6 a	26.6 a
150-210	50.7 a	42.1 a	49.2 a	51.6 a	30.2 a	36.3 a	37.9 a	31.3 a
			Sprin	g sampling				
0-30	44.1 c	14.7 a	32.3 bc	31.2 b	30.6 a	23.9 a	34.3 a	30.8 a
30-60	18.6 b	4.89 a	8.7 a	19.5 b	13.6 a	10.4 a	15.8 a	14.1 a
60-90	10.7 b	4.7 a	7.98 ab	21.3 с	13.3 a	9.0 a	11.7 a	12.9 a
90-120	11.3 b	6.50 a	10.2 ab	19.7 c	11.5 a	8.6 a	11.7 a	13.2 a
120-150	17.0 b	9.6 a	13.9 ab	24.2 c	14.9 a	10.5 a	12.4 a	16.4 a
150-180	15.1 a	11.8 a	15.3 a	23.2 b	15.2 a	10.6 a	16.6 a	16.7 a
180-210	17.5 a	15.6 a	17.2 a	25.9 b	18.0 a	11.1 a	14.4 a	19.9 a
30-90	29.3 b	9.54 a	17.1 a	40.8 c	26.9 a	19.5 a	27.6 a	27.0 a

90-150	28.3 b	16.1 a	24.3 ab	43.9 с	26.4 a	19.2 a	24.1 a	29.6 a
150-210	29.7 a	25.0 a	30.0 a	45.4 b	30.4 a	19.1 a	27.6 a	33.4 a

Table 22. Fall and spring sum of NO₃-N (kg ha⁻¹) and NH₄-N (kg ha⁻¹) from 0-90 cm and from 0-210 cm deep for 14 farms, and cover crop biomass (kg ha⁻¹), N content (kg N ha⁻¹), and C/N ratio for 19 farms. Cover crop treatment values for a response variable, within the same season and farm, followed by the different letters are significantly different (p < 0.05); * indicates significantly different (p < 0.1).

				Cove	r crop						So	oil			
		Late	e-fall samp	oling	Sp	ring sampl	ing		Late-fall	sampling			Spring s	ampling	
		Bioma	N	C/N	Bioma	N	C/N	NO) ₃ -N	NI	I ₄ -N	NO	O ₃ -N	NI	I ₄ -N
		SS	content	ratio	SS	content	ratio	0-90	90-210	0-90	90-210	0-90	90-210	0-90	90-210
								cm	cm g ha ⁻¹	cm	cm	cm	cm	cm	cm
	Radish	6298 a	82.4 a	27.2 a	NA	NA	NA	18.6 a	24.0 a	86.5 a	84.8 a	14.1 ab	14.2 a	32.0 a	25.9 a
Dorches	Wheat	1529 b	29.2 b	21.1 b	814 a	10.8 a	22.5 a	16.2 a	23.8 a	62.7 a	61.5 a	5.93 ab	8.63 a	40.3 a	29.9 a
ter IB ¹	Mix	4509 c	61.0 c	27.5 a	1047 a	12.0 a	28.0 a	12.3 a	24.0 a	63.3 a	77.5 a	3.40 a	16.2 a	28.9 a	20.7 a
	Contro 1							22.8 a	21.1 a	43.2 a	51.4 a	23.0 b	12.1 a	35.3 a	25.5 a
	Radish	2829 a	48.1 a	22.8 a	NA	NA	NA					46.0 ab	44.9 a	66.0 a	94.9 a
Frederic	Tritica le	1194 b	23.8 b	21.1 a	1263 a	26.2 a	18.7 b	-				26.9 a	50.6 a	58.4 a	57.1 a
k I	Contro 1	2371 c	47.8 a	19.7 a	1210 a	28.0 a	15.7 a	36.2 a	49.9 a	31.5 a	22.6 a	31.1 a	38.7 a	57.0 a	54.6 a
	Contro 1							58.2 a	63.9 a	25.9 a	21 a	60.3 b	58.7 a	99.5 a	118 a
	Radish											31.9 ab	93.0 a	84.4 a	122 a
Frederic k III	Tritica le				1183 a	30.3 a	15.9 a					14.5 a	90.1 a	43.2 a	64.5 a
	Mix				1079 a	30.3 a	13.3 b					19.2 a	116 a	39.4 a	48.7 a

	Contro 1											57.9 b	161 a	100 a	170 a
	Radish	2182 a	53.9 a	15.2 a	NA	NA	NA	35.1 a	39.1 a	83.7 a	56.5 a				
Frederic k IV ²	Tritica le	1140 b	31.3 a	15.1 a	4799	55.5	36.8	40.0 a	69.4 b*	94.3 a	128 a				
	Contro 1							84.6 b	47 ab	98.7 a	119 a				
	Radish	3477 a	79.5 a	18.1 a				36.6 a	149 a	43.0 a	30.5 a				
Harford I	Mix	1967 a	50.5 a	16.2 a	1	1		38.6 a	69.7 a	40.6 a	27 a				
	Contro 1							85.0 b	76.1 a	42.0 a	26.8 a				
	Radish	2269 a	45.9 a	19.1 a	NA	NA	NA	31.3 a	14.6 a	31.4 a	5.05 a				
Howard IA ³	Rye	1158 b	28.9 b	16.8 b	1345	29	19.5								
	Contro 1							77.3 a	10.1 a	30.0 a	9.15 a				
	Radish	1964 a	46.3 a	13.1 a	NA	NA	NA					56.4 с	50.3 a	140 a	173 a
Howard	Rye	1150 b	26.9 b	16.5 b	3165 a	41.2 a	30.5 b					16.1 a	37.7 a	70.7 a	85.0 a
IB	Mix	2095 a	56.1 a	13.7 ab	3571 a	68.2 b	21.7 a					27.7 ab	52.4 a	143 a	188 a
	Contro 1											38.8 b	55.9 a	83.3 a	94.6 a
	Radish	3717 a	122 a	12.0 a	NA	NA	NA	59.6 a	208 a	62.0 a	86 a	163 b	172 ab	62.7 a	38.4 a
Huntingt on IA	Rye	2315 b	83.2 a	12.1 a	4533	126	14.7	104 a	166 a	132 a	137 a	39.9 a	110 a*	34.7 a	26.2 a
	Mix	3864 a	132 a	11.7 a	NA	NA	NA	146 a	234 a	81.9 a	88.7 a	225 c	165 ab	68.6 a	47.3 a

	Contro 1							178 a	254 a	63.4 a	80.3 a	194 bc	292 b	44.0 a	62.9 a
	Radish	2380 a	37.3 a	23.4 a	NA	NA	NA					41.5 a	5.43 a	21.0 a	4.9 a
	Rye	1055 b	20.5 b	20.1 b	1325 a	15.8 a	34.9 a					22.1 a	6.85 a	27.0 a	7.73 a
Kent II ⁴	Mix	2039 ab	36. 6 a	21.0 ab	2271 b	25.4 a	37.3 a					12.8 a	7.00 a	92.0 a	46.0 a
	Late- mix	1216 b	26.5 ab	16.4 c	2244 b	27.7 a	34.1 a					33.9 a	20.5 a	76.5 a	50.6 a
	Contro 1							-				28.9 a	23.3 a	21.5 a	12.9 a
	Radish							99.8 ab	82.3 a	61.9 a	56.7 a	77.4 ab	21.9 a	30.8 a	17.5 a
Lancaste	Tritica le	4333	115	15.8	3988	87.5	18.9	60.5 a*	67.0 a	36.6 a	30.9 a	20.9 a	8.43 a	23.8 a	14.6 a
r IA ⁵	Mix							92.2 ab	81.2 a	61.9 a	69.4 a	73.0 ab	19.4 a	34.9 a	34.8 ab
	Contro 1							129 b	80.8 a	31.9 a	18.5 a	126 b	83.4 b	38.0 a	37.3 b*
	Radish	4162 a	86.9 a	17.3 a	NA	NA	NA	-				72.7 a	34.0 a	80.6 a	37.3 a
	Tritica le	1291 b	40.1 b	13.1 bc	7321 a	60.0 a	52.4 b					16.8 b*	36.0 a	50.3 a	42.2 a
Lancaste r IB	Mix	2240 b	54. 7 ab	15.7 ab	7459 a	88.9 a	36.8 a					35.3 ab	25.6 a	92.9 a	33.5 a
	Late- mix	1249 b	38.54 b	11.1 c	9479 a	174 a	28.1 a	-				26.3 ab	36.2 a	62.2 a	16.5 a
	Contro 1											67.8 a	74.9 b	79.5 a	31.1 a
Lancaste	Radish	5712 a	202 a	11.1 a	NA	NA	NA	33.1 a	58.8 a	20.3 a	10.9 a	58.0 ab	42.9 ab	18.1 a	14.2 a
r II	Tritica le	3607 a	126 a	12.1 a	3014 a	88.8 a	14.0 b	36.5 a	76.2 a	27.1 a	31.8 b	24.8 a	23.8 a	22.8 a	9.87 a

	Mix	4900 a	165 a	12.2 a	1503 b	51.4 b	11.8 a	51.1 a	67.9 a	47.7 b	37.1 b	47.8 ab	36.8 ab	19.4 a	13.0 a
	Contro 1							125 b	54.8 a	16.6 a	10.7 a	79.9 b	54.3 b	23.8 a	13.1 a
	Radish											64.1 a	31.7 a	49.4 a	40.8 a
Lancaste	Tritica le				2619 a	53.9 a	20.4 b					36.2 b	17.0 b	62.6 a	29.7 a
r III ⁶	Mix				2572 a	67.1 a	16.1 a					30.1 b	16.0 b	45.4 a	25.9 a
	Contro 1											54.4 ab	30.5 a	63.5 a	44.3 a
	Radish	4915 a	186 a	8.50 b	NA	NA	NA	55.2 a	60.6 a	33.5 a	6.83 a	183 a	111 a	26.3 a	11.7 a
Lancaste	Tritica le	3448 b	148 b	9.14 ab	7125 a	202 a	14.0 b	74.2 a	71.5 a	35.7 a	8.28 a	44.0 b	55.1 b	33.2 a	13.3 a
r V	Mix	5326 a	204 a	9.4 a	1830 b	68.3 b	10.6 a	70.0 a	76.1 a	60.7 a	31.3 a				
	Contro 1							280 b	116 b*	25.6 a	8.1 a	81.7 b	127 a	24.6 a	19.0 a
	Radish	1330 a	48.0 a	10.8 a	NA	NA	NA								
Franklin I	Tritica le	837 a	29.2 a	12.0 a	2620 a	68.4 a	15.5 b								
	Mix	1359 a	42.0 a	13.2 a	1653 a	48.8 a	13.9 a								
	Radish	3359 a	117 a	10.9 a	NA	NA	NA								
Franklin IIA	Oat	1848 b	57.4 b	14.9 a	NA	NA	NA								
	Mix	2853 ab	82.5 ab	14.5 a	NA	NA	NA								
	Radish	900 a	31.0 a	10.2 a	NA	NA	NA								

Franklin	Oat	569 b	23.1 a	9.3 a	NA	NA	NA	 	 		 	
IIB	Mix	977 a	38.3 a	9.8 a	NA	NA	NA	 	 		 	
	Radish	3023 a			NA	NA	NA	 	 		 	
Huntingt on IB	Ryegr ass	1567 a						 	 		 	
	Mix	2240 a						 	 		 	
	Radish	222 a	7.76 a	10.3 b	NA	NA	NA	 	 		 	
Prince	Rye	146 a	4.85 ab	12.9 a	826 a	15.0 a	23.0 a	 	 		 	
Georges I	Wheat				1024 a	17.0 a	25.5 a	 	 		 	
	Mix	128 a	3.53 b	11.1 ab	921 a	14.3 a	26.1 a	 	 		 	

Dorchester IB soil was sampled only to 180 cm for fall and spring samples.
 Frederick IV soil was sampled only to 180 cm in fall.
 Howard IA soil was sampled only to 120 cm in fall.
 Kent II soil was sampled only to 180 cm in spring.
 Lancaster IA soil was sampled only to 180 cm in spring.
 Lancaster III soil was sampled only to 180 cm in spring.

Table 23. Cover crop biomass (kg ha⁻¹), N content (kg N ha⁻¹), and C/N ratio for fall cover crop growth (late-fall sampling), fall and spring cover crop growth (spring sampling), and cover crop growth prior to termination (late-fall radish, prior to winter-kill, and spring winter cereal, prior to herbicide termination. Different letters indicate statistically significant differences between cover crop treatments.

Cover crop treatment	N	Biomass (kg ha ⁻¹)	N content (kg ha	C/N ratio							
_		_	1)								
	La	ate-fall sampling ¹									
Radish	13	3085 a	85.5 a	14.2 a							
Winter cereal	13	1586 с	52.1 b	14.2 a							
Mixed species	13	2651 b	77.6 a	14.5 a							
Spring sampling ²											
Winter cereal	10	3046 b	61.3 a	23.2 b							
Mixed species	10	2385 a	49.9 a	19.2 a							
S	amplir	ng prior to termination	on^3								
Radish before termination	13	2663 a	79.7 a	13.4 a							
Winter cereal before	13	3026 a	61.6 b	22.6 b							
termination											

¹ Values reported for late-fall sampling from 13 farms—Franklin I, Howard IB, Lancaster II, Prince Georges I, Lancaster V, Franklin IIA, Franklin IIB, Lancaster IB, Huntington IA, Huntington IB, Dorchester IB, Frederick I, Kent II.

² Values reported for spring sampling from 10 farms—Franklin I, Howard IB, Lancaster II, Prince Georges I, Lancaster V, Lancaster IB, Frederick III, Lancaster III, Frederick I, Kent II.

³ Values reported for sampling prior to termination from 13 farms—Franklin I, Franklin IIA, Franklin IIB, Frederick I, Frederick IV, Howard IA, Howard IB, Huntington IA, Kent II, Lancaster IB, Lancaster II, Lancaster V, and Prince Georges I). On Franklin IIA and Franklin IIB, the winter cereal (oat) naturally winter-killed rather than being chemically terminated.

Table 24. Correlations among cover crop biomass and growing degree days (GDD), precipitation (prec.) total from cover crop planting date to sampling date, topsoil (0-30 cm) NO₃-N and NH₄-N (kg N ha⁻¹), topsoil percent sand/clay/silt. Table showing number of replicates (N), correlation coefficient (r), and p-value of correlation.

Biomass		GDD	Prec.	NO ₃ -N	NH ₄ -N	% sand	% clay	% silt
Fall winter cereal	N	16	16	14	14	14	14	14
	r	0.288	0.184	0.774	0.039	-0.521	0.224	0.517
	p-value	0.28	0.494	0.001	0.895	0.056	0.442	0.058
Fall radish	N	16	16	14	14	14	14	14
	r	0.433	0.233	0.484	0.118	-0.467	-0.011	0.558
	p-value	0.094	0.385	0.080	0.688	0.092	0.970	0.038
Spring winter cereal	N	15	15	15	15	15	15	15
	r	0.629	0.576	0.400	-0.271	-0.744	0.438	0.703
	p-value	0.012	0.025	0.140	0.328	0.002	0.102	0.004

Table 25. June PSNT soil sample NO₃-N and NH₄-N concentrations (mg N kg⁻¹ soil), and corn plant biomass per corn plant (g plant⁻¹) and N in biomass per corn plant (g N plant⁻¹) following cover crop treatments. N indicates the number of replicates per cover crop treatment. Cover crop treatment values for a response variable, within the same farm, followed by the different letters are significantly different (p < 0.05).

Site				CEREAL			CONTROL
	NO ₃ -N	3	10.56 a	14.15 a	5.80 a		10.4a
Dorchester IB	NH4-N	3	5.39 a	7.37 a	7.38 a		7.80 a
	Corn biomass	3	1.19 a	0.609 b	0.862 ab		1.10 ab
	Corn N	3	0.0531 a	0.0283 b	0.0395 ab		0.0479 ab
	NO ₃ -N	4	6.53 a	7.02 a	8.03 a		5.11 a
Franklin IIB	NH ₄ -N	4	6.77 a	7.11 a	4.98 a		5.72 a
	Corn biomass	4	3.25 a	2.67 a	2.38 a		2.41 a
	Corn N	4	0.114 a	0.0965 a	0.0904 a		0.0894 a
	NO ₃ -N	4	13.11 a	7.32 a			11.43 a
Frederick IV	NH4-N	4	0.923 a	1.124 a			0.724 a
	Corn biomass	4	3.15 a	1.50 b			3.23 a
	Corn N	4	0.115 a	0.0557 b			0.116 a
	NO ₃ -N	4	5.37 a	2.09 b	3.46 ab		3.30 ab
Howard IB	NH4-N	4	5.19 a	7.16 a	7.24 a		10.36 a
	Corn biomass	4	7.00 a	3.32 с	5.72 b		5.38 b
	Corn N	4	0.234 a	0.090 c	0.172 b		0.158 b
	NO ₃ -N	3	8.00 a	4.66 ab	3.56 b	3.78 b	6.46 ab
Kent II	NH4-N	3	8.15 a	7.16 a	7.93 a	6.44 a	8.11 a
	Corn biomass	3	3.01 a	2.13 a	2.30 a	2.51 a	2.50 a
	Corn N	3	0.129 a	0.0781 a	0.0887 a	0.0997 a	0.102 a
	NO ₃ -N	4	19.4 a	7.24 a	10.6 a	11.5 a	11.7 a
Lancaster IB	NH4-N	4	10.2 a	9.20 a	11.09 a	9.71 a	9.22 a
	Corn biomass	4	5.58 a	3.51 b	3.78 b	4.38 ab	5.29 a
	Corn N	4	0.246 a	0.155 b	0.175 b ¹	0.201 ab	0.236 a
	NO ₃ -N		10.6 a	6.87 b	6.79 b		8.04 ab
6 Farms	NH ₄ -N		6.04 a	6.45 a	6.64 a		6.90 a
	Corn biomass	84	4.02 a	2.37 с	3.03 b		$3.46 b^2$
	Corn N	84	0.154 a	0.0867 с	0.113 b		0.129 b

¹ Difference between a and b significant at p < 0.055.

² Difference between a and b significant at p < 0.056.

Table 26. Percent of maximum corn yield following cover crop treatments for farmers' standard fertilizer application rate (standard) or no fertilizer application. Cover crop treatment values for percent of maximum corn yield, within the same fertilizer N level, followed by different letters are significantly different (p < 0.05).

Site	N rate, kg ha ⁻¹	Radish	Winter cereal	Mix	Control						
		Percent of maximum yield									
6 farms ¹	Standard ²	92% a	71% c	77% bc	86% ab						
6 farms ³	0 fertilizer ⁴	85% a	56% с	73% b	73% b						

¹ Farms include Frederick IV (corn silage) and Howard IA, Howard IB, Frederick I, Lancaster IA and Lancaster IB (corn grain)

² Rate that farmer normally uses

³ Farms include Franklin IIB (corn silage) and Howard IA, Howard IB, Frederick I, Lancaster IA and Lancaster IB (corn grain)

⁴ Lancaster IB applied 67 kg N ha⁻¹ at planting

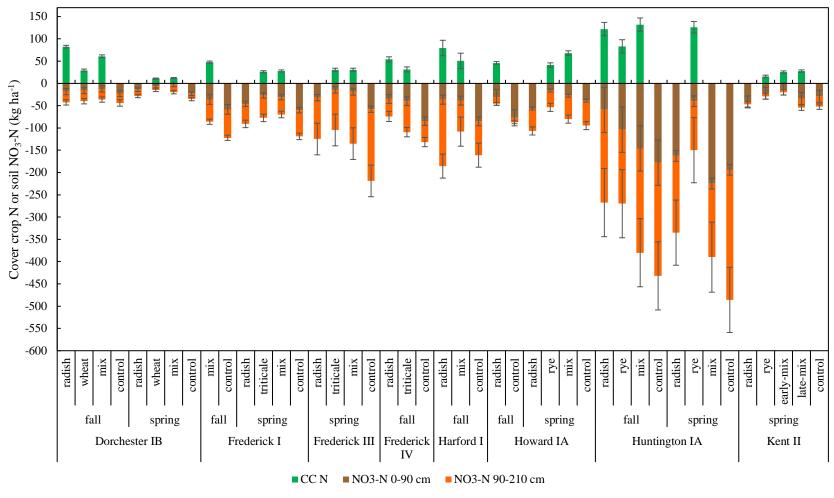


Figure 15. Cover crop N uptake (CC N; green bars) and soil NO₃-N (kg ha⁻¹) from 0-90 cm (brown bars) and 90-210 cm (orange bars) for 13 farms with biomass and soil samples collected. Belowground N (soil) indicated with negative values. Standard error bars show.

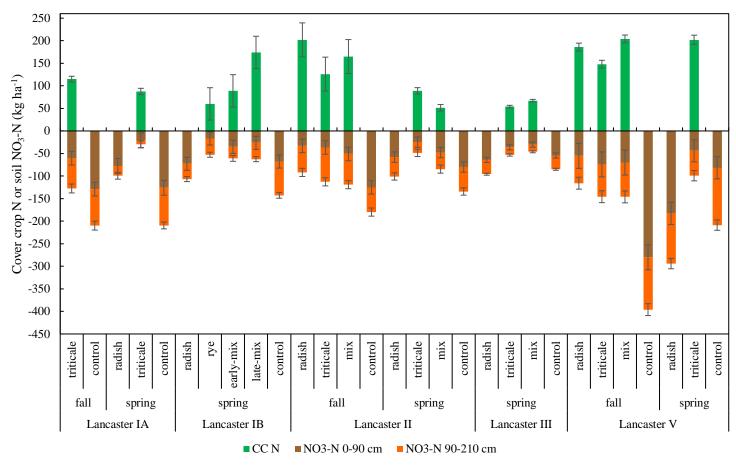


Figure 15 continued

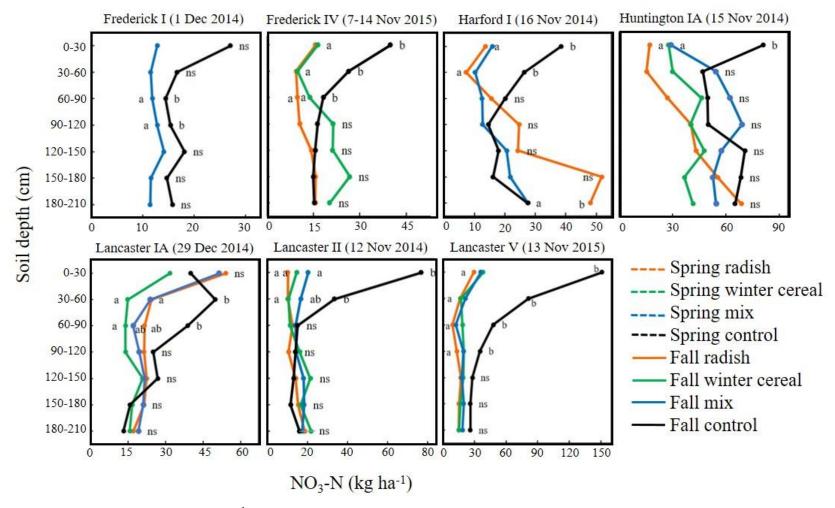


Figure 16. Amount of NO_3 -N (kg ha^{-1}) in 0-210 cm soil profile for seven farms at fall sampling and 10 farms at spring sampling. ¹Spring samples from 120-150 cm and 150-180 cm depths are the average values from 120-180 cm.

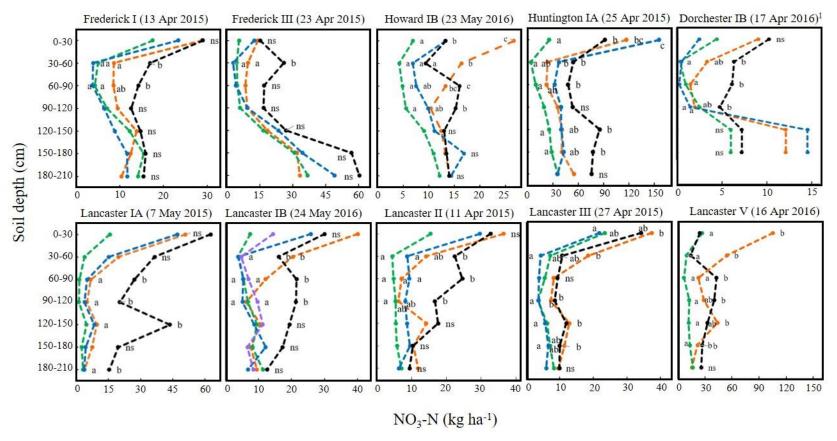


Figure 16 continued

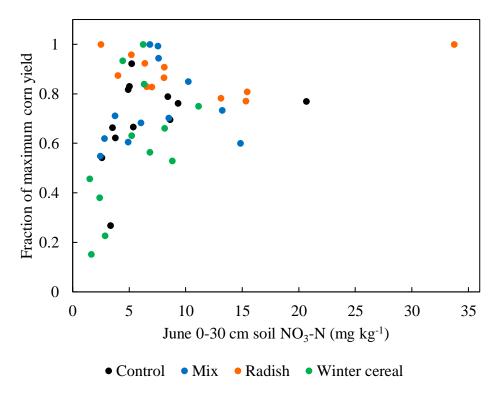


Figure 17. Relationship between fraction of maximum corn yield (no N applied on corn) and pre-sidedress test nitrate concentrations for cover crop treatments. Data from Howard IB, Franklin IIB, and Lancaster IB.

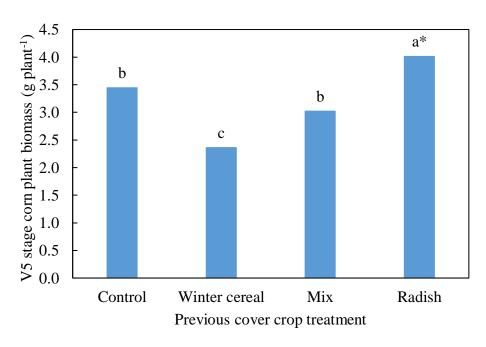


Figure 18. Corn biomass at V5 growth stage for cover crop treatments. Corn biomass values with different letters are significantly different (p < 0.05). Data from Dorchester IB, Frederick IV, Howard IB, Franklin IIB, Kent II and Lancaster IB. *Differences between radish and control at p < 0.0561.

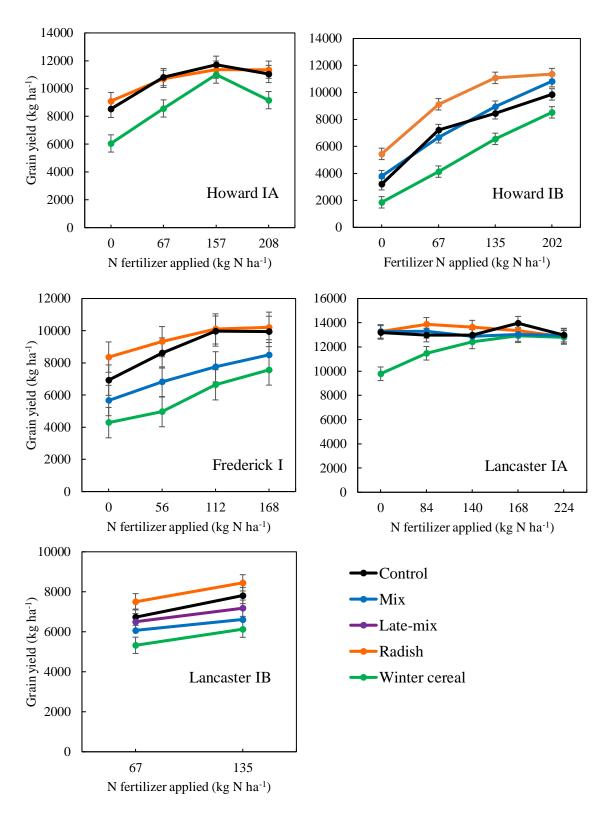


Figure 19. Corn grain yield associated with various N fertilizer rates and preceding cover crop treatments. Error bars show standard error of mean.

Appendix 7. Supplemental soil characteristics of study sites

Table 27. Study site soil pH, percent sand, percent clay, percent C, and percent N for each 15 or 30 cm soil depth increment (0-210 cm), and percent soil organic matter (SOM), P, K, Mg, Ca, and S (mg kg⁻¹) for the upper 30 cm of soil from each farm site. Each record is the average of two to three composited soil cores from two areas in the field. Data from Dorchester IB 180-210 cm and Lancaster IB 180-210 cm is from a single point of a field. Soil samples were not taken from Franklin IIA or Huntington IB; however these sites were within 100 meters of Franklin IIB and Huntington IA, respectively. Values not determined indicated as nd. Values below detection limit indicated as BDL.

Farm site	Depth	pH ¹	Sand ²	Clay ²	C ³	N³	SOM ⁴	P ⁴	K ⁴	Mg ⁴	Ca ⁴	S ⁴
5100	cm				%	l	l			ppm	l	I
	0-30	6.0	34.9	12.8	0.805	0.071	2.25	47.5	43	75. 5	643	20
Frankl in IIB	30-60	5.2	35.4	20	0.17	0.022						
Dorch	60-90	4.5	20.1	19.6	0.16	0.027						
	90-120	4.8	45.7	17.6	0.15	0.025						
ID	120-150	4.9	59.2	15.3	0.146	BDL						
	150-180	5.1	62.9	15.2	0.13	0.020						
	180-210	6.0	27.1	26.9	0.181	0.033						
	0-15	5.5	45.9	18.9	0.943	0.092	2.35	19	69	127	777	18.5
	15-30	5.9	46.5	22.4	0.458	0.051	1.65	9.5	56	122	718	15.5
	30-45	nd	nd	nd	0.309	0.037						
	45-60	5.8	39	32.7	0.393	0.046						
	60-75	nd	nd	nd	0.183	0.028						
	75-90	6.5	29.3	40.6	0.153	0.028						
	90-105	nd	nd	nd	0.232	0.034						
in I	105-120	5.8	29.2	40.5	0.177	0.028						
	120-135	nd	nd	nd	0.211	0.031						
	135-150	4.8	26.7	27.9	0.22	0.031						
	150-165	nd	nd	nd	0.202	0.032						
	165-180	4.9	21.7	29.4	0.118	0.026						
	180-195	nd	nd	nd	0.131	0.027						
	195-210	4.9	28.3	26.2	0.152	0.025						
	0-30	6.8	17.9	22	1.258	0.120	2.9	49	80. 5	111	142 1	3.5
Frank!	30-60	6.7	11.4	33.2	0.588	0.064						
	60-90	6.6	18.4	30.9	0.441	0.049						
	90-120	5.9	30.6	47.8	0.303	0.044						
	120-150	5.6	27.9	59.9	0.179	0.048						

	150-180	5.1	17.6	54	0.141	0.043						
	180-210	5.0	13.9	56.8	0.152	0.045						
	0-15	6.4	38.5	16.5	1.097	0.118	2.75	80	71. 5	125	948	12
	15-30	6.2	41.1	17.4	0.536	0.060	1.85	24.5	48	101	929	12.5
	30-45	nd	nd	nd	0.319	0.046						
	45-60	6.2	36.3	28.6	0.224	0.036						
	60-75	nd	nd	nd	0.15	0.033						
	75-90	5.2	32	30.9	0.119	0.024						
Freder	90-105	nd	nd	nd	0.13	0.027						
ick I	105-120	4.9	42.7	24.1	0.131	0.030						
	120-135	nd	nd	nd	0.183	0.034						
	135-150	4.8	50.1	11.3	0.082	BDL						
	150-165	nd	nd	nd	0.077	BDL						
	165-180	4.7	47.2	11.4	0.064	0.020						
	180-195	nd	nd	nd	0.081	0.023						
	195-210	4.9	49.8	10.4	0.082	BDL						
	0-15	6.9	29.1	24.7	1.498	0.181	4.3	87.5	83	93	180 2	15
	15-30	6.7	26.8	32.3	0.606	0.102	2.4	20	47	97. 5	137 5	8.5
	30-45	nd	nd	nd	0.258	0.079						
	45-60	6.5	23.9	37.4	0.202	0.077						
	60-75	nd	nd	nd	0.142	0.075						
	75-90	6.0	19.7	35.5	0.116	0.073						
Freder ick III	90-105	nd	nd	nd	0.094	0.068						
ick III	105-120	5.2	18.5	34.3	0.086	0.063						
	120-135	nd	nd	nd	0.174	0.077						
	135-150	4.9	23.8	29	0.082	0.067						
	150-165	nd	nd	nd	0.077	0.069						
	165-180	4.9	24.4	30.1	0.072	0.061						
	180-195	nd	nd	nd	0.079	0.065						
	195-210	4.6	30.7	26.2	0.071	0.061						
	0-30	7.3	29.1	22.5	1.025	0.105	2.2	69.5	73. 5	108	147 0	3.5
	30-60	7.4	18.7	40	0.285	0.041						
Freder	60-90	7.4	18.5	48.5	0.223	0.043						
ick IV	90-120	6.6	21.6	46.2	0.163	0.038						
	120-150	6.7	28.3	38.7	0.137	0.033						
	150-180	7.1	26.5	45.9	0.169	0.044						
	180-210	7.7	28.5	36.7	1.557	0.039						

	0-15	6.1	26.6	19.5	1.738	0.159	4.3	17	95	133	728	11
	15-30	5.7	24.9	25.6	0.76	0.078	2.3	4	59	99	543	29
	30-45	nd	nd	nd	0.433	0.051						
	45-60	5.3	29.5	23.3	0.204	0.032						
	60-75	nd	nd	nd	0.157	0.024						
	75-90	5.1	38.5	16.9	0.107	BDL						
Harfor	90-105	nd	nd	nd	0.083	BDL						
d I	105-120	5.2	36.3	14.7	0.099	0.020						
	120-135	nd	nd	nd	0.258	0.032						
	135-150	5.2	40.5	9.9	0.118	0.026						
	150-165	nd	nd	nd	0.093	BDL						
	165-180	5.2	44.2	8.1	0.1	0.022						
	180-195	nd	nd	nd	0.056	BDL						
	195-210	5.1	43.2	13.2	0.071	0.021						
	0-15	6.5	43.9	12.6	2.193	0.214	4.2	60.5	126	111	170 5	17.5
	15-30	6.5	47.7	15.7	1.126	0.103	2.6	36.5	59. 5	104	146 4	16.5
	30-45	nd	nd	nd	1.297	0.108						
	45-60	6.8	25.5	32.6	1.423	0.111						
	60-75	nd	nd	nd	1.324	0.097						
	75-90	6.9	35.3	27.6	0.883	0.062						
Howa rd IA	90-105	nd	nd	nd	0.682	0.047						
	105-120	6.6	35.9	10.2	0.724	0.050						
	120-135	nd	nd	nd	0.758	0.058						
	135-150	6.8	47.7	24.9	0.438	0.030						
	150-165	nd	nd	nd	0.374	0.028						
	165-180	6.7	48.3	18.6	0.363	0.023						
	180-195	nd	nd	nd	0.229	0.020						
	195-210	7.0	68.9	12.3	0.256	BDL						
	0-30	6.2	47.8	16.7	0.703	0.071	2.1	16.5	51. 5	76	571	16
	30-60	6.5	50.7	17.2	0.484	0.048						
Howa	60-90	6.4	29.2	23.8	0.364	0.039						
rd IB	90-120	6.1	23.7	28.3	0.207	0.034						
	120-150	5.7	30.5	23.9	0.134	0.027						
	150-180	5.9	42	19.8	0.139	0.023						
	180-210	5.9	39.9	19.3	0.157	0.021						
Hunti ngton	0-15	6.5	27	20.6	2.829	0.228	5.4	93.5	228	277	137 8	8.5
IA	15-30	6.0	24.3	25.5	0.792	0.076	2.6	23.5	127	164	680	22.5

	30-45	nd	nd	nd	0.369	0.050						
	45-60	4.8	19.2	38	0.213	0.038						
	60-75	nd	nd	nd	0.163	0.033						
	75-90	4.7	22.7	35.1	0.155	0.033						
	90-105	nd	nd	nd	0.217	0.034						
	105-120	4.6	39.7	28.3	0.117	0.027						
	120-135	nd	nd	nd	0.668	0.062						
	135-150	4.7	18.9	46.3	0.146	0.039						
	150-165	nd	nd	nd	0.178	0.029						
	165-180	4.6	28.1	46	0.13	0.033						
	180-195	nd	nd	nd	0.099	0.034						
	195-210	4.5	16.3	49.4	0.174	0.038						
	0-15	6.0	45.8	8.2	1.015	0.101	2.65	27.5	72	58	495	2
	15-30	5.7	44.5	10.1	0.509	0.056						
	30-45	nd	nd	nd	0.292	0.036						
	45-60	5.9	36.3	17.9	0.222	0.038						
	60-75	nd	nd	nd	0.167	0.033						
	75-90	5.8	49.3	17.7	0.12	0.027						
Kent	90-105	nd	nd	nd	0.067	0.022						
IIB	105-120	5.6	85	5.6	0.052	0.015						
	120-135	nd	nd	nd	0.268	0.030						
	135-150	5.1	84	3.5	0.052	BDL						
	150-165	nd	nd	nd	0.05	BDL						
	165-180	5.3	91.4	3.7	0.038	BDL						
	180-195	nd	nd	nd	0.031	BDL						
	195-210	4.8	92.6	2.7	0.035	BDL						
	0-15	6.7	29.2	20.6	1.952	0.202	4.7	87	154	116	140 1	29.5
	15-30	6.3	33.1	22.6	0.953	0.098	3.1	33.5	47. 5	84	841	7.5
	30-45	nd	nd	nd	0.286	0.039			-			
	45-60	6.4	35.3	27.3	0.414	0.049						
Lanca	60-75	nd	nd	nd	0.176	0.029						
ster IA	75-90	6.4	44.9	23.3	0.09	BDL						
	90-105	nd	nd	nd	0.102	BDL						
	105-120	6.3	52.8	18.2	0.079	BDL						
	120-135	nd	nd	nd	0.266	0.038						
	135-150	5.9	55.4	17.6	0.095	0.022						
	150-165	nd	nd	nd	0.078	BDL						

	165-180	5.5	55.5	17.2	0.06	BDL						
	180-195	nd	nd	nd	0.061	BDL						
	195-210	5.4	62.5	11	0.044	BDL						
	0-30	6.1	30.5	25.2	0.869	0.087	3.45	81.5	38	60	763	13.5
	30-60	6.2	38.6	29.9	0.292	0.034						
Lanca	60-90	6.1	51.5	26.5	0.167	0.021						
ster	90-120	5.7	55.1	18.4	0.095	BDL						
IB	120-150	6.1	62.3	15.6	0.14	BDL						
	150-180	6.0	67.4	8.3	0.084	BDL						
	180-210	5.7	73.7	4.4	0.054	BDL						
	0-15	6.7	32.2	16.5	1.654	0.184	3.95	104	120	130	159 9	45.5
	15-30	6.8	31	21.3	1.035	0.123	3.1	54.5	81. 5	107	135 6	37.5
	30-45	nd	nd	nd	0.331	0.053						
	45-60	6.7	39	16.9	0.264	0.048						
	60-75	nd	nd	nd	0.094	0.029						
	75-90	6.8	50.7	7	0.075	0.026						
Lanca ster II	90-105	nd	nd	nd	0.056	0.023						
Ster II	105-120	6.8	53.7	3.1	0.036	BDL						
	120-135	nd	nd	nd	0.153	0.036						
	135-150	6.8	60.1	2.3	0.037	0.025						
	150-165	nd	nd	nd	0.029	0.023						
	165-180	6.7	63.4	2.3	0.027	0.027						
	180-195	nd	nd	nd	0.028	0.025						
	195-210	6.9	57.2	2.7	0.029	0.023						
	0-15	6.4	46.6	12.7	1.484	0.164	3.15	101	185	207	118 7	2
	15-30	6.5	49	14.8	0.847	0.096	2.1	59.5	137	155	940	4
	30-45	nd	nd	nd	0.337	0.046						
	45-60	5.8	47.6	16	0.519	0.060						
	60-75	nd	nd	nd	0.52	0.060						
Lanca	75-90	5.9	45.7	18.7	0.335	0.045						
ster III	90-105	nd	nd	nd	0.267	0.040						
	105-120	5.2	43.9	17.9	0.158	0.028						
	120-135	nd	nd	nd	0.204	0.032						
	135-150	4.9	51	17.9	0.114	0.022						
	150-165	nd	nd	nd	0.084	0.020						
	165-180	5.3	50.3	17.3	0.095	0.020						
	180-195	nd	nd	nd	0.113	0.021						

	I	1		1	1	1	1	1	ı	1	ı	ı
	195-210	5.4	44.9	15.4	0.116	BDL						
	0-30	7.1	10.7	17.1	1.581	0.153	2.75	120	134	196	162 6	12.5
	30-60	6.8	14	24.4	0.56	0.055						
Lanca	60-90	7.0	13.9	23.3	0.352	0.034						
ster V	90-120	7.1	17.2	23.6	0.223	0.025						
	120-150	7.0	30.9	22	0.186	0.021						
	150-180	6.8	26.2	26.4	0.132	BDL						
	180-210	7.1	24.7	32	0.254	0.030						
	0-15	5.2	59.7	11.9	1.416	0.128	2.9	61	144	165	633	14
	15-30	5.2	56.5	16	0.657	0.068	1.85	42	112	152	643	9.5
	30-45	nd	nd	nd	0.55	0.063						
	45-60	4.9	43.4	30.6	0.357	0.052						
	60-75	nd	nd	nd	0.193	0.037						
	75-90	4.6	55.7	23.9	0.15	0.034						
Prince	90-105	nd	nd	nd	0.151	0.033						
Georg es I	105-120	4.5	69.6	20	0.187	0.031						
	120-135	nd	nd	nd	0.353	0.044						
	135-150	4.5	69.6	20.1	0.12	0.028						
	150-165	nd	nd	nd	0.103	0.025						
	165-180	4.4	78.3	14.9	0.103	0.023						
	180-195	nd	nd	nd	0.091	0.020						
	195-210	4.4	77.6	13.6	0.104	0.022						

¹ pH by glass combination pH electrode and a pH meter (Metler Toledo InLab®413 combination meter).
² Soil particle size analysis by the Modified Pipette Method. Gavlak, R., D. Horneck and R.O. Miller. 2005. Particle size analysis modified pipette method. Soil, plant and water reference methods for the western region. 3rd ed.

³ Total C and N analysis at University of Maryland Department of Environmental Science and Technology Analytical Lab on LECO CN628 Elemental Analyzer (LECO Corp., St. Joseph, MI; Nelson and Sommers, 1996: Mateiovic, 1993)

^{1996;} Matejovic, 1993).

⁴ Soil organic matter (SOM) (Loss On Ignition method) and nutrient content by Mehlich3 extraction (P, K, Mg, Ca, Na, S) at WayPoint Analytical, Inc (Richmond, VA).

Appendix 8. Cover crop sampling details

Table 28. Fall and spring cover biomass number and size of quadrats collected from each

plot.		
Farm	Fall quadrats	Spring quadrats
Franklin I	2 x (0.25 m2)	2 x (0.25 m2)
Franklin IIA	2 x (0.25 m2)	NA
Frederick I	2 x (0.25 m2)	2 x (0.25 m2)
Frederick III		2 x (0.25 m2)
Harford I	2 x (0.25 m2)	
Howard IA	3 x (0.25 m2)	3 x (0.25 m2)
Huntington IA	2 x (0.25 m2)	2 x (0.25 m2)
Lancaster IA	2 x (0.25 m2)	2 x (0.25 m2)
Lancaster II	2 x (0.25 m2)	2 x (0.25 m2)
Lancaster III		2 x (0.25 m2)
Prince Georges I	2 x (0.25 m2)	2 x (0.25 m2)
Franklin IIB	3 x (0.25 m2)	
Frederick IV	5 x (0.25 m2)	3 x (0.25 m2)
Howard IB	3 x (0.25 m2)	3 x (0.25 m2)
Huntington IB	2 x (0.50 m2)	
Kent II	4 x (0.25 m2)	3 x (0.25 m2)
Dorchester IB	4 x (0.25 m2)	3 x (0.25 m2)
Lancaster IB	3 x (0.25 m2)	3 x (0.25 m2)
Lancaster V	5 x (0.25 m2)	3 x (0.25 m2)

Appendix 9. List of weather stations

Table 29. Distance from weather station to farm for precipitation and temperature measurements.

	Distance to w	eather station
Farm	Precipitation	Temperature
	k	m
Caroline I	5.86	35.7
Dorchester IA	8.01	11.9
Franklin I	4.80	4.80
Franklin IIA	4.85	4.85
Frederick I	5.83	18.6
Frederick III	0.89	22.9
Harford I	13.7	13.7
Howard IA	5.20	9.78
Huntington IA	7.09	27.7
Kent I	17.0	30.4
Lancaster IA	7.99	7.99
Lancaster II	2.20	9.31
Lancaster III	7.03	28.0
Prince Georges I	4.43	4.43
Dorchester IB	1.61	12.1
Franklin IIB	4.64	4.64
Frederick IV	3.69	21.9
Howard IB	6.03	9.27
Kent II	11.3	19.1
Lancaster IB	10.1	10.1
Lancaster V	5.06	5.06

Appendix 10. Bulk density of soil cores

The number of cores that were averaged and the depth increments varied by farm (Table 30). The bulk density values for each layer from each farm were analyzed using box and whisker plots in SAS version 9.4 (SAS Institute, Cary, NC). To exclude outliers and possible errors, values beyond 1.5 x the interquartile range (25th to 75th percentiles) were not included when calculating the average bulk density value.

Table 30. Bulk density (BD) mean (g cm $^{-3}$), standard deviation (SD) (g cm $^{-3}$), first (Q1) and third (Q3) interquartile range (25th to 75th percentiles) and number of outliers above fences for all farms in which soil cores were taken. The fence is defined as 1.5 x

Interquartile range (25th to 75th percentiles).

							# of outliers
	Soil depth,	# of cores	BD mean,	BD SD, g			above
Site	cm	averaged	g cm ⁻³	cm ⁻³	BD Q1	BD Q3	fences
Dorchester IB	0-60	36	1.502	0.125	1.43	1.59	1
	60-120	36	1.72	0.213	1.60	1.87	1
	120-180	35	1.53	0.297	1.37	1.81	1
Frederick I	0-120	29	1.63	0.272	1.54	1.66	1
	120-210	25	1.87	0.227	1.71	2.02	1
Frederick III	0-120	20	1.22	0.187	1.12	1.37	1
	120-210	19	1.48	0.359	1.24	1.74	1
Frederick IV	0-60	24	1.39	0.0780	1.33	1.47	1
	60-120	23	1.54	0.138	1.46	1.63	1
	120-210	23	1.38	0.275	1.28	1.62	1
Harford I	0-120	15	1.31	0.273	1.16	1.49	1
	120-210	15	1.19	0.362	1.02	1.44	1
Howard IA	0-120	9	1.43	0.0881	1.35	1.49	1
	120-210	6	0.985	0.281	0.782	1.28	1
Howard IB	0-60	25	1.31	0.0634	1.26	1.34	1
	60-120	25	1.48	0.110	1.38	1.57	1
	120-210	27	1.51	0.280	1.38	1.64	1
Huntington IA	0-120	32	1.27	0.385	0.864	1.59	1
	120-210	30	1.39	0.427	1.02	1.69	1
Kent II	0-60	24	1.53	0.124	1.44	1.62	1
	60-120	24	1.62	0.0936	1.58	1.66	1
	120-210	23	2.08	0.512	1.56	2.65	1
Lancaster IA	0-120	29	1.22	0.217	1.08	1.43	1
	120-210	28	1.48	0.240	1.35	1.67	1
Lancaster IB	0-60	26	1.14	0.180	1.01	1.23	1
	60-120	25	1.39	0.175	1.28	1.49	1
	120-210	26	1.44	0.312	1.25	1.57	1

Lancaster II	0-120	32	1.05	0.158	1.01	1.15	1
	120-210	32	1.12	0.179	1.06	1.26	1
Lancaster III	0-120	20	1.36	0.263	1.23	1.54	1
	120-210	18	1.57	0.341	1.34	1.75	1
Lancaster V	0-60	40	1.30	0.0814	1.25	1.36	1
	60-120	40	1.63	0.115	1.57	1.70	1
	120-210	38	1.50	0.323	1.35	1.70	1

Appendix 11. Corn yield equations

Equation 19 Corn silage yield

$$Silage\ yield = \frac{whole\ plant\ dry\ wt}{harvest\ area}$$

Where,

whole plant dry weight = whole plant wet wt *
$$\frac{(100-whole plant \% moist.)}{100}$$

whole plant % moisture =
$$\left(1 - \frac{\text{subsample dry plant} + \text{ear weight}}{\text{subsample wet plant} + \text{ear weight}}\right) * 100$$

Harvest area = row width x length of harvest area

Equation 20 Corn grain yield

$$Grain\ yield = \frac{grain\ weight\ at\ 15.5\%\ moist.}{harvest\ area}$$

Where,

$$grain\ weight\ at\ 15.5\%\ moist. = wet\ ear\ wt * \frac{subsample\ dry\ grain\ wt}{subsample\ dry\ ear\ wt} * \frac{15.5}{ear\ \%\ moist.}$$

$$ear \% moisture = \left(1 - \frac{dry \ ear \ wt}{wet \ ear \ wt}\right) * 100$$

Chapter 5: Policy implications and Conclusion

Policy Implications—improving efficiency of cover crop program through deep rooted cover crops

Currently the State of Maryland has an incentive program in which landowners are paid to grow cover crops. The incentive payment amounts vary, depending on cover crop species, cover crop planting date, previous cash crop, and field management practices. Farmers are eligible for cover crop payments if they plant the cover crop by 5 Nov and kill after 28 Feb. The program gives "early planting" bonus payments if the cover crop is planted before 15-October (Maryland Department of Agriculture, 2018). We found in the current study that cover crops planted after 30-September will have minimal biomass accumulation and soil N uptake and will not capture subsoil N in the fall. Under the current cover crop program, planting rye alone is given a bonus incentive over planting rye within a mix. However, we found in the current study that rye monocultures typically require additional spring N fertilization or decrease subsequent corn yields. If planting cover crops leads to increased N fertilizer requirements, it is counterproductive toward the goal of using cover crops to reduce residual soil N and risks of N leaching from cropland.

The EPA Interim Evaluation of Maryland's 2016-2017 milestones reports that the Agriculture sector in Maryland was not on-track to reach its 2017 N target, which is a 60% reduction of the 2009 N loads into the Bay to achieve water quality standards (EPA, 2017). While this failure may be partly a result of legacy N effects due to the slow flow of groundwater (Ator and Denver, 2015), it may also be partly a result of conservation

practice implementation. For example, cover crops will not perform to their full potential if they are planted too late. Due to the extent of cover crops on the landscape (e.g., 478,000 acres in Maryland in 2014), improvements in the ability of cover crops to reduce N leaching from the land through incorporating earlier planting dates and more deeprooted species could foster a more sustainable, cycling crop system and greatly reduce the N load into bodies of water. We therefore suggest that incentives be increased for earlier cover crop planting, especially for planting prior to mid-September. We recognize that such early cover crop planting may require additional adaptations of a farm system, such as earlier maturing crop varieties, interseeding into standing crops, or changes in crop rotations.

Overall conclusions

We found there were often trade-offs between scavenging residual N and releasing the N for the subsequent crop. Radish was very effective at scavenging N in the fall; however, it sometimes led to increased levels of NO₃-N in the spring soil in shallow as well as deep soil layers (e.g., Lancaster III and Lancaster V farms in Figure 16). The ¹⁵N tracer study indicated that radish, rye, and two-way or three-way mixes of radish + rye + (crimson clover) all performed equally well in scavenging residual N from deep soil layers. Winter cereal cover crops caused a yield loss and/or increased N fertilization needs for a subsequent corn crop.

Utilizing mixed cover crop species may be optimal in terms of N scavenging and release. Mixed cover crops were very effective at scavenging residual N in the fall and spring, and typically did not cause a yield gain or loss for subsequent crops. Radish may

also be a good choice, in terms of being able to reduce subsequent corn N fertilizer application amounts.

The effect of cover crops on the cropping system may take more than one growing season to become apparent. For example, the phenomenon observed on Lancaster IA, where there was no corn yield response to N fertilizer, may have resulted from the long-term cumulative effects of many years of mixed-species cover cropping and manure applications on this farm. Furthermore, it is important to keep in mind that cover crop effects are expected to be highly variable from site-to-site and year-to-year, according to soil and weather patterns. Thus, while the on-farm proponent of our study was not as precisely controlled as the research station trials, we believe it was very valuable for considering the range of cover crop responses that we would expect to see across practitioners.

In conclusion, we found substantial levels of inorganic soil N remained in the soil profile (0-210 cm deep) following summer crops. On average, there was more residual inorganic N in the soil profile than the amount of N fertilizer that a farmer would typically apply to a corn crop. This provides both a risk and an opportunity. The residual N is at risk to leach into bodies of water and cause eutrophication and associated environmental problems. However, the pool of residual N also could serve as a valuable resource to farmers, if they utilize it and reduce their fertilizer use. Cover crops were able to access deep pools of N, but only if the cover crops were planted by the first week in September in our study region. If planted early, forage radish and winter cereal cover crops were very effective at scavenging deep soil N from 1+ m deep and in some cases even up to 2 m deep. The radish cover crop was sometimes effective at recycling N to

subsequent crops. We expect radish and mixed species may have greater positive effects on subsequent crops after several years of use.

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