BRASSICA COVER CROPS FOR NITROGEN RETENTION IN THE MARYLAND COASTAL PLAIN

by

Jill Elise Dean

ABSTRACT

Title of thesis: BRASSICA COVER CROPS FOR NITROGEN RETENTION IN THE MARYLAND COASTAL PLAIN

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The Brassica cover crops, forage radish (*Raphanus sativus* L. cv 'Daichon'), oilseed radish (*Raphanus sativus* L. cv 'Adagio'), rape (*Brassica napus* L. cv 'Dwarf Essex'), and cereal rye (*Secale cereale* L. cv 'Wheeler') were examined for ability to decrease mineral N losses and influence organic N cycling at two Maryland Coastal Plain agricultural sites. Brassicas were similar or superior to rye regarding N uptake and soil profile NO_3-N depletions (105-180 cm depth). Rape and rye maintained soil porewater NO_3-N below 3 mg L^{-1} throughout spring while radish performed similarly on fine-textured soil, but caused porewater NO_3-N > 10 mg L^{-1} on coarse-textured soil. Dissolved organic N averaged 51% of total N in porewater, but was unaffected by cover crops. Brassicas were as effective as rye in minimizing mineral N losses, but the role of cover crops in managing organic N was unclear.

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May 2005. Negative values indicate a loss. Small letters indicate significantly different means within column, $p<0.1$.

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CHAPTER I

INTRODUCTION

BACKGROUND AND PROBLEM DEFINITION

Agricultural losses of nitrate and organic nitrogen (N) are contributing to the degradation of surface waters mainly from agricultural sources. In 1998, the Maryland General Assembly passed the Water Quality Improvement Act to help combat the eutrophication of surface waters and contamination of drinking water. The Water Quality Improvement Act requires management strategies for the reduction of nutrient inputs into surface waters. Acknowledging the specific contribution of agricultural inputs, the Maryland Cover Crop Program was established to encourage farmers to grow cover crops during the fall and winter months after the harvest of the summer crop and before the planting of the next cash crop. Subsidies are paid to farmers who use certain cover crops in their nutrient management plans and the subsidy amount is dependent on the planting date of the cover crops.

The fall and winter months typically have a higher incidence of precipitation and lower evapotranspiration due to the lack of plants (post-harvest) or the dormancy of plants. Decreased evapotranspiration and nutrient uptake by plants may contribute to wetter soils during these cooler months and nutrients may be carried outside of the rooting zones of the summer crops by mass flow of the soil solution and slowly transported to surface waters (Campbell et al. 1994). Leaching potential is also influenced by soil texture, fertilizer inputs, soil drainage class, and N transformations (Campbell et al. 1994).

Cover crops grown in the fall and winter aid in the control of nutrient pollution and balancing nutrient budgets by capturing nutrients leftover from the fertilization of summer crops, storing the nutrients within the plant tissues of the cover crops, and increasing evapotranspiration to reduce the quantity of soil solution and nutrients leaching through soil (Kuo et al. 1995; McCracken et al. 1994; Weinert et al. 2002; Meisinger et al. 1991). As the cover crops die and decomposition occurs, the nutrients are released back into the soil for use by future crops if the residues are not removed from the site. The continual use of the nutrients by plants throughout the year conserves the nutrients, keeping them within the plant-soil system, and decreases the quantity of nitrate and other forms of nitrogen lost from the system due to leaching. Cover crops decrease leaching losses with fewer negative effects on the following cash crop yields compared to decreased fertilizer inputs (Gustafson et al. 2000).

Nitrate is at a high risk for leaching loss in the mid-Atlantic region due to repulsion by negatively-charged soil particle surfaces. Precipitation, coupled with lack of plant capture and decreased evapotranspiration, enables large quantities of anionic nutrients such as nitrate to be lost from plant-soil ecosystems.

Organic forms of N are now being examined for the role they may play in surface water impairment in addition to the effects of nitrate inputs. Dissolved organic N (DON) has been shown to positively impact the populations of dinoflagellates, pelagophytes, and cyanobacteria—several species of these groups contributing to harmful algal blooms (Paerl 1988; Berg et al. 1997; LaRoche et al. 1997; Lomas et al. 2001; Glibert et al. 2001; Glibert et al. 2004). At this time, it is unclear what role, if any, cover crops may play in the cycling of organic N

The small grain crops rye and barley and the Brassica rapeseed are subsidized by the Maryland Cover Crop Program at this time. Research indicates that these cover crops work well for reducing N losses in the Maryland Coastal Plain, the area where nutrient pollution is most sensitive due to the proximity of farms to the Chesapeake Bay and its watershed. Studies performed in Europe, Canada and the western United States suggest that other Brassica cover crops, forage and oilseed radishes, may also effectively control N losses in the mid-Atlantic region. Rapeseed, forage and oilseed radishes are deep-rooted and quickly grow through the soil when planted by early fall to scavenge the deeper subsoil stores of N that are at high risk for leaching beyond the rooting zone (Thorup-Kristensen 2001). The suitability of Brassicas such as forage and oilseed radishes should be investigated to determine the possible benefits to farmers in the Maryland coastal plain.

Justification for Research

A need for profitable means of nutrient management and nutrient loss reductions remains in Maryland. Farms may feel burdened by the cost of cover crop seed, extra fertilizer and pesticides as well as the extra labor involved with the use of cover crops since these crops do not generally increase capital. While cover crops themselves are not sold, they may provide benefits that increase the profitability of the following cash crops.

Brassica cover crops may provide multiple benefits to farmers in the Mid Atlantic Region. Studies indicate that Brassicas may impact nematode populations and suppress plant predatory nematodes (Gardner and Caswell-Chen 1993; Halbrendt, 1992), suppress weeds through the release of allelopathic compounds (Petersen et al 2001), reduce subsoil compaction (Materechera et al. 1992), and decrease nitrogen leaching losses (Thorup-Kristensen 2001).

Brassica crops typically maintain a fleshy taproot and deep rooting depths are attained more quickly than small grains such as rye (Thorup-Kristensen 2001) if planted by early fall (Vos and Van der Putten, 1997). The quick growth of the rooting system would suggest efficient capture of leaching N compounds and transfer to plant shoot tissue. Thorup-Kristensen (2001) observed that N uptake from the deeper soil layers had a greater impact on N leaching losses than N uptake from surface layers. For those Brassicas that winterkill—forage and oilseed radishes, the decomposition and release of this once-subsoil N at the soil surface from the decay of the aboveground plant material acts as a biological pump for nutrients. The N deposited by the Brassicas at the soil surface may then be used for the nutrient requirements of the following summer crop, decreasing the need for fertilizer applications. Those Brassicas that do not winterkill such as rape continue to capture subsoil N and decrease its leaching potential throughout the winter and spring.

Rye is an established and efficient cover crop, but Brassica cover crops such as rape, forage and oilseed radishes remain unexamined in their potential for N uptake and leaching reductions in the Mid-Atlantic Region. Studies including Brassicas indicate that they are at least as efficient in N conservation as cereal rye, and may be more effective at scavenging deep N due to their extensive rooting systems and rooting depth (Thorup-Kristensen 2001; Kristensen and Thorup-Kristensen, 2004). Overwintering crops such as rape and rye are somewhat more effective at depleting subsoil N reserves than those crops that winterkill (Weinert et al., 2002) however because they continue to capture N into the spring. Investigations may find Brassicas' are well-suited to the Mid Atlantic

Region climate and for helping achieve Maryland's goals for reducing nutrient additions to the Chesapeake Bay.

General Research Approach

Field experiments were conducted between August 2003 and May 2005 at three sites within the Maryland coastal plain to assess cover crop N uptake and the influence of cover crops on residual soil N in fall and spring, and on N leaching losses throughout winter and spring. The experiments compared a no cover (winter weeds) control treatment. to several cover crops, including forage radish (*Raphanus sativus* L. cv. 'Daichon'), oilseed radish (*Raphanus sativus* L. cv. 'Adagio'), rapeseed (*Brassica napus* L. cv. 'Dwarf Essex'), and the small grain rye (*Secale cereale* L. cv. 'Wheeler').

Plant N uptake was quantified by harvesting of cover crop shoots at near-maximum fall growth and again prior to planting the following summer main crop. The fleshy taproots of the radishes and rape were also harvested. Residual soil N was quantified by taking soil cores to a depth of 120-180 cm depth using a Veihmeyer corer (Veihmeyer, 1929). Cores were analyzed in 15 cm increments for extractable ammonium, nitrate and organic forms of N. Tension lysimeters were installed to depths of 75-120 cm and used to sample soil porewater weekly in late winter and early spring and analyzed for nitrate-N and total soluble nitrogen (TSN) concentrations.

General Research Hypotheses and Objectives

Research Hypotheses

The research was designed to test these overall hypotheses:

1. Brassica N uptake is greater than or equal to that of rye in the fall

2. Subsoil layers under the brassicas will contain less soluble mineral N and soluble organic N (SON) than under no cover crop plots and rye plots
3. Soil pore water will contain less NO_3-N and dissolved organic N (DON) under Brassica crops than under no cover crop and rye plots

Research Objectives

In order to test the above hypotheses, research was conducted with these objectives in mind:

1. Quantify N uptake by forage radish (*Raphanus sativus* L. cv. 'Daichon'), oilseed radish (*Raphanus sativus* L. cv. 'Adagio'), rape (*Brassica napus* L. cv. 'Dwarf Essex') and rye (*Secale cereale* L. cv. 'Wheeler') to capture NO_3-N and NH_4-N
2. Quantify ability of these cover crops to remove mineral N and soluble organic N (SON) from deep in the soil profile before it can leach to groundwater
3. Quantify the leaching losses of NO_3-N and DON under the aforementioned cover crops
4. Compare effects of the Brassica cover crops to that of rye and no cover crop controls.

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Chapter II

Literature Review

INTRODUCTION

The eutrophication and subsequent decrease in biodiversity (Hale et al., 2004) in areas of the Chesapeake Bay are attributed mainly to the influx of nitrogen and phosphorus pollution from nonpoint sources such as agriculture and urban areas, and point sources such as wastewater treatment facilities. Human terrestrial activity has a large affect on the Chesapeake Bay because the land area within the Chesapeake Bay basin is large (94% of basin) relative to the water surface area (6% of basin) and stored water volume in the Bay (2410 km^2/km^3) (Pionke et al., 2000). Nutrient inputs to surface water may result in degradation of drinking water, health hazards to humans and livestock (Dorsch et al., 1984; Nielsen and Lee, 1987), and decreased economic productivity of the Bay. According to the Chesapeake Bay Program (2005), 39% of nitrogen pollution to the Bay is agricultural in origin. Nitrogen commonly reaches the Bay by leaching through soil to groundwater although atmospheric deposition following ammonia volatilization, denitrification, and erosive losses may also contribute to the N load. During the fall, winter, and spring when agricultural fields lay fallow, the potential for nitrogen pollution is at its highest. Cover crops grown during this time may decrease N leaching (Jackson et al., 1993; Vos and Van der Putten, 2001; Weinert et al., 2002; Meisinger et al., 1991).

Acknowledging the efficiency of cover crops for reducing N leaching, the Maryland Department of Agriculture established a Cover Crop Program in June 2003. Farmers who apply to the program may now be paid subsidies for growing crops such as rye and rape during the winter months. In 2003, 116,710 acres of cover crop were planted on the one million acres of cropland in Maryland with cost share support by the

Cover Crop Program (Maryland Department of Agriculture, 2005). Rye is a very popular winter cover in Maryland due in part to its adaptability to different soils but also its nitrogen scavenging and recycling ability. Brassica cover crops such as rape (*Brassica napus* L.), forage radish (*Raphanus sativus* L.) and oilseed radish (*Raphanus sativus* L.) are recent introductions to the Atlantic coastal plain and are currently being investigated for their N uptake potential in the Maryland coastal plain.

Mineral N (NH_4^+ plus NO_3^-) is considered to be the main form of N from agricultural sources to impact surface waters. The solubility of nitrate and its low retention in soils due to the repulsion of anions by negative soil surface charges allow it to be transported from the soil and transferred to groundwater. Ammonium may be found in surface runoff and can also be converted to nitrate by soil microflora. Denitrification is not a large contributor to N losses during the fall-spring seasons (Isse et al., 1999) due to colder temperatures and subsequent reduced microbial activity required for the transformation of NO_3^- to nitrogen oxide species and dinitrogen gas (N_2).

Recent evidence suggests that organic N may also contribute to eutrophication and water quality impairment. Christou et al. (2005) found dissolved organic nitrogen to be 57± 8% of the total dissolved nitrogen pool averaged over 94 sites with 7 contrasting agricultural management schemes. Qualls et al. (1991) found organic N to comprise 94% of the dissolved N leaching through a deciduous forest soil. Significant portions of dissolved organic N may be taken up directly by biota or mineralized in freshwater (Brookshire et al., 2005) and further aggravate eutrophication in bodies of water. Some evidence suggests that plants and phytoplankton take up organic forms of N (Okamoto and Okada, 2004; Glibert et al., 2004; Nasholm et al., 1998; Jones et al., 2004) therefore even if portions of organic N are not mineralized they may still have direct ecological impacts.

Winter cover crops, or catch crops, reduce the likelihood of nitrate leaching by absorbing and storing nitrogen within plant tissues, and decreasing the amount of water that moves through the soil column by plant water uptake and evapotranspiration (Weinert et al., 2002; Meisinger et al., 1991). Infiltration is also increased in soil under cover crops which diminishes the N lost through surface runoff although it may increase the possibility of soil N leaching into groundwater. The suitability of a species as a catch crop is enhanced by rapid leaf expansion, partitioning of dry matter to roots, rapid root growth, and the ability of the species to sequester nutrients in its tissues throughout the leaching season (Vos and Van der Putten, 1997). Cover crops not only sequester N within their tissues, but also release N for the summer crop as the cover crop residues decompose. Decomposition of cover crop residue may therefore increase both the organic matter and nitrogen concentrations in the soil (Hargrove, 1986; Kuo et al., 1995; McVay et al., 1989; Meisinger et al., 1991; Wilson et al., 1982). When comparing cover crops to reduced fertilizer applications as a means of decreasing N losses, cover crops were more effective and had fewer consequences for the following main crop yield (Gustafson et al., 2000).

To decrease nutrient loading to bodies of water, cover crops must be efficient scavengers of N. The primary method of sequestering N is by absorbing N directly from the soil and then storing the N in plant tissues. Consequently, N uptake usually parallels biomass production with larger N accumulation in those plants that produce the most biomass. Variations in plant response to sowing date and quickness of establishment also play major contributions in the decrease of soil N (Vos and Van der Putten, 1997; Thorup-Kristensen, 2001).

Overwintering cover crops—those that are not killed by cold temperature in the winter months—such as rapeseed, cereal rye, and wheat, are generally more effective at reducing subsoil NO_3-N than frost-killed crops even if the shoot N uptake is similar (Weinert et al., 2002). Those plants that demonstrate rapid germination, and aggressive, extensive rooting systems are also effective as N scavengers (Sainju et al. ,1998). Cereals and Brassica cover crops exhibit these characteristics and have been shown to be a complementary addition to cover crop systems (Wagger and Mengel, 1988; Staver and Brinsfield, 1998; Shipley et al., 1992; Coale et al., 2001; Meisinger et al., 1991; Thorup-Kristensen, 2001; Kristensen and Thorup-Kristensen, 2004).

ROOTING SYSTEMS OF COVER CROPS INFLUENCE NITROGEN CAPTURE

Nitrogen fertilization beyond the needs of the summer main crop may result in a substantial amount of nitrate movement below the rooting zone. Length of fallowing time, amount of precipitation, and soil properties may also have a significant influence on the amount of nitrate that is leached past the root zone (Campbell et al., 1984; Campbell et al., 1994). Once below the root zone, N encounters few obstacles to leaching to groundwater and being slowly transported to surface waters. Thus it is the deep soil N that is most susceptible to leaching loss. Extensive rooting in deep soil layers may therefore reduce leaching losses (Meisinger et al., 1991) more than roots that take up comparable amounts of nitrogen from the upper soil layers (Thorup-Kristensen, 2001; Thorup-Kristensen and Nielsen, 1998). Brassicas have been found to reduce subsoil N more effectively than monocots such as rye (Aufhammer et al., 1992; Thorup-Kristensen, 1994).

Kristensen and Thorup-Kristensen (2004) found root growth to be correlated with nitrate uptake in deep soil horizons for rye, ryegrass, and forage radish. Planted on August 8, winter rye and forage radish were both found to have average rooting depths greater than 1 m, but the radish reached this depth by September 20 (43 days after planting) while rye did not until October 16 (69 days after planting). By November 3 (87 days after planting), the rooting depth for rye was 1.15 m and 2.27 m for forage radish. Soil NO_3-N under the rye was 4 times higher than under forage radish over the depth of soil to 2.5 m. From 0-1.0 m depth, the soil NO_3-N was almost 3 times higher under rye than radish. Using minirhizotrons, it was also established that the root intensity and root frequency of the forage radish was significantly greater than both rye and ryegrass to the depth of 2.5 m.

The ability of cover crops to prevent leaching is influenced by the growth rate of the plants, seasonal variation, and the sowing date—all of which may be intimately related to rooting systems. For a short growing season (planting later than September), cereals may have the advantage of faster initial root growth over the cruciferous crops (Thorup-Kristensen, 2001; Vos and Van der Putten, 1997) when temperatures are cooler. Crucifers, however, develop a deeper rooting system when planted earlier in the fall (August through mid-September) due to faster rooting depth penetration in warmer temperatures and are beneficial with longer growing seasons (Thorup-Kristensen, 2001; Kristensen and Thorup-Kristensen, 2004).

Vos and Van der Putten (1997) noted a decreased response to N with delay in sowing date for cereal rye (*Secale cereale*) and rape (*Brassica napus* ssp. *oleifera*) with rape being more sensitive to sowing date and accumulating less N than rye during shorter fall growing seasons. However, in the spring the difference in N response was found to be insignificant since the rye was less winter-hardy than rape and lost some of its previously stored N due to frost damage to tissues (Vos and Van der Putten, 1997). Later sowing dates have a negative effect on the amount of dry matter produced and the N uptake for both species, especially rape. Nitrogen uptake was much less affected by late fall weather conditions for the rape in the treatment with a late August sowing date (S1) than for rye.

Table 1 summarizes several studies using brassica, rye, and ryegrass cover crops to reduce N leaching. In the studies of Thorup-Kristensen (2001), Isse et al. (1999), and Vos and Van der Putten (2001), cover crops significantly reduced soil NO_3 concentrations and N leaching potential. In the Kristensen and Thorup-Kristensen (2004) study, forage radish (*Raphanus sativus* L. var. *oleiformis* Pers.) significantly reduced soil NO_3 more than rye and ryegrass from 0-2.5 m. Weinert et al. (2002) found that white mustard (*Brassica hirta* Moench,'Martagena') was not as effective a N scavenger as rape (*Brassica napus* L. "Jupiter") or rye (*Secale cereale* L.). Most studies show that, compared to bare fallow control treatments, forage radish, rape, and rye decrease soil NO_3 to similar extent in the upper 1 m of soil. The study that analyzed the deepest soil layers, down to 2.5 m, reported that forage radish reduced significantly more N from the 1-2.5 m depths than did rape or rye (Kristensen and Thorup-Kristensen 2004). Few studies were to be found that measured soil N contents below 1.0 m.

Vos and Van der Putten (2001) reported (Table 1) similar amounts of N in the upper 1 m of soil in early spring whether cover crops were used or not. The cover crops were planted on August 19 and soil NO_3-N was sampled on March 16 just before killing the crops. After planting in August 19, 70 kg N ha^{-1} were added to all treatments. Vos and Van der Putten explain the similar amounts of NO_3-N left in the soil under the rape, rye, and control on March 16 by stating that the 51 kg N ha^{-1} in the control is what remained after leaching losses throughout the fall and winter, whereas the 47 and 49 kg N ha^{-1} under the rye and rape plots, respectively, are what has been conserved by those crops through plant N uptake. Both above and below ground plant tissues were sampled on March 16 and rye and rape captured 123 and 138 kg N ha^{-1}, respectively. The exact depth to which root matter was collected was not stated. Vos and Van der Putten did not report any fall soil NO_3-N results to differentiate the abilities of the crops to take up soil N compared to the control.

PLANT NITROGEN UPTAKE CAPABILITIES

Plant nitrogen uptake capacity is the product of tissue N concentration and biomass production. Unlike gramineous cover crops, brassicaceous cover crops have a fleshy storage root that provides a significant amount of biomass and potential nitrogen storage above what is stored in the aboveground tissues**.** Nitrogen is thereby sequestered in plant tissues during the winter and spring when evapotranspiration is low and leaching potential is greatest. Some cover crops are winterhardy, like cereal rye and rape, and will store N until killed in spring at which time removal of aboveground tissues or application to the soil surface as a green manure may occur. Other species such as forage and oilseed radishes are frostkilled and release their N during the early spring. Nitrogen uptake in nonlegumes is directly affected by biomass production since their tissues have a small range of N concentrations (Holderbaum et al., 1990; Wagger and Mengel, 1988).

Table 2 summarizes the biomass and N uptake measurements of several studies using various Brassicas, rye and ryegrass. Shoot and root biomass production for the Brassicas (average of 3744 and 1156 kg ha^{-1} for radish shoots and roots, 2720 kg ha^{-1} and 544 kg ha^{-1} rape and canola shoots and roots) is similar if not greater than the monocots (3124 and 1298 kg ha^{-1} shoots and roots) even though the rooting structure is very different between the 2 groups. Forage radish, oilseed radish, rape (*Brassica napus L.),* and rye have similar shoot to root ratios (2.1-8.4) while canola (*Brassica rapa L.)* produces much greater amounts of shoot biomass compared to root matter (13 and 22). Cereal rye has become a popular cover crop in the coastal plain region due to its proven efficacy in N uptake (Shipley et al. 1992; Staver and Brinsfield 1998) and the Brassicas may also prove to be as effective in N scavenging for the region.

If at all possible, roots should be sampled and analyzed for a more complete study of nutrient uptake and management. The fleshy Brassica taproot is much easier to extract from soil and to clean than is the fibrous root system of rye leading to omission of rye root data or insufficient data in many studies (Mokany et al., 2006).

Reduction of Mineral Nitrogen in Groundwater

Nitrate is extremely mobile within soil systems. Obviously precipitation, irrigation, or winter snow melting would greatly enhance nitrate mobility in a soil. A water application of 2.5 cm is capable of leaching nitrate 19 cm further into a coarse-textured soil at field capacity according to Endelman et al. (1974). Tyler et al. (1992) and Bundy and Andraski (2005) observed leaching losses up to 50 kg NO_3-N ha^{-1} after large rainstorms that occurred following fertilization. Dynamics of soil NO_3-N content to a depth of 30 cm was found to be related to rainfall amounts after fertilization (Bundy and Andraski, 2005). Weil et al. (1990) measured losses of 71-167 kg NO_3-N ha^{-1} in irrigated, coarse textured soils between October and March.

Plant cover reduces nitrogen leaching by uptake of N into the plant and by decreasing soil water volume through transpiration (Merbach et al., 1993; Justes et al., 1999; Logsdon et al., 2001; Weinert et al., 2002; Bundy and Andraski, 2005). Fall planted cover crops help to reduce NO_3-N leaching losses in the coastal plain, which has favorable conditions for N mineralization through October although the main crop may be harvested earlier in fall (Staver and Brinsfield, 1998).

Staver and Brinsfield (1998) measured mineral N reductions in leaching water and on the underlying aquifer after several years of rye cover crop addition to long-term continuous corn rotation. Lysimeters installed to 60 cm depth in plots and wells were used to monitor leachate and water table N concentrations. Rye was introduced into the crop rotation in 1998 and from introduction until 1997, groundwater NO_3-N decreased from almost 30 to ~4 mg L^{-1}. Staver and Brinsfield postuled that even less cold-tolerant cover crop species might be as effective as rye with early fall planting and moderate to low soil NO_3-N amounts following the harvest of the main crop.

Merbach et al. (1993) performed a lysimeter study using ^{15}N fertilizer applied to oilseed radish and no cover control in growth chambers with simulated weather conditions for late fall-early winter. After application of the approximate of 500 kg N ha^{-1} in a field study, leachate was collected for 20 day intervals from the lysimeters. After 80 days, oilseed radish leaching losses of ^{15}N (63.4 kg ha^{-1} over 80 days) were 77% less than that of control (218 kg ha^{-1}).

Thorup-Kristensen (2001) estimated from soil samples taken from the 1-1.5 m depth that NO_3 in the soil water at this same depth ranged from 119 µg/L under the control plots to 23 and 1.5 µg/L in the rye and forage radish plots respectively. Unfortunately, this is not a direct measurement of the soil water N.

Isse et al. (1999) directly measured soil water NO_3-N at a depth of 55 cm using zero-tension lysimeters from November to May on forage radish, ryegrass, and control plots. Gravitational water nitrate content (GWNC) samples were taken at 2 sites, Ste. Rosalie and St. Bernard, only 4 times during the winter leaching period from January through May however. While the lysimeters may hold a month's worth of porewater, the dynamics of the system are then lost to the average of the month. Isse's leaching data is weak because only a few samples were obtained over a long period of time. Isse et al. estimated 215-236 mm of percolating water per year at Ste. Rosalie and St. Bernard, and calculated a loss of 30 kg N ha^{-1} yr^{-1} from control plots and 1.2-27 kg N ha^{-1} maximum losses under cover crops at Ste. Rosalie. At St. Bernard, 64-76 kg N ha^{-1} yr^{-1} were estimated lost from control plots and 30-55 kg N ha^{-1} maximum losses under cover crop plots due to leaching processes.

Without knowledge of the soil profiles in the sites used by Isse et al (1999), discussion of whether the depth of the lysimeters was adequate is difficult, but it seems that a deeper placement of the lysimeters in addition to more frequent sampling would have resulted in a more conclusive experiment. From previous literature on rooting depths of the brassicas, depths of 90-120 cm for lysimeter placement would seem to more accurately measure the crops' ability to reduce leaching potentials. The lysimeters were only sampled when soil water was seen in the lysimeter tubes. It is unclear how long the sample would have been in the lysimeter before removal. It is inaccurate to state that the GWNC was from a certain date since the water may have been accumulating in the lysimeters for several days before it was actually sampled, and nitrogen transformations may also have occurred depending on the biotic and abiotic processes, pH of the soil solution, and temperature within the lysimeters.

ORGANIC NITROGEN

Dissolved organic N (DON) and soluble organic N (SON) make up the pool of organic N in soil. Dissolved organic N (DON) is defined as the organic forms of N found in soil solution and in soil leachate (Murphy et al., 2000). Soluble organic N (SON) is the organic N that can be extracted from the soil using water or salt solution (Murphy et al., 2000). Both DON and SON are composed of similar classes of organic N, but differ in the proportions of those classes. Amino acids, amino sugars and larger phenolic compounds make up the organic N pool, but soil pH and functional groups such as carboxyls and hydroxyls determine which compounds sorb to soil (Kaiser and Zech, 2000; Vieublé Gonod et al., 2006; Vinolas et al., 2001) to become SON and which remain DON in the soil solution. Dissolved organic N encompasses a wide range of compounds, mostly hydrophilic (Lehman et al., 2004; Tipping et al., 1999), from low molecular weight (LMW) amino acids and amino sugars that are easily mineralizable by microorganisms and some plants (Mulvaney et al., 2001; Jones et al., 2004) to recalcitrant phenolic compounds (Jones et al., 2004; Smith, 1987). Biodegradability of DON decreases as LMW amino compounds are consumed and DON inputs to marine waters were observed to be resistant to microbial degradation (Peierls and Paerl, 1997; Stepanauskas et al., 1999). Soluble organic N has a higher proportion of easily mineralizable amino acids, amino sugars and humic N compounds (Mengel et al., 1999; Dick et al., 2000) usually adsorbed to mineral horizons (Tipping et al., 1999; Kaiser et al., 1997). SON reservoirs may be as large as mineral N pools (Jensen et al., 1997; Bhogal et al., 2000) in agricultural soils, but DON averages about 10% of the SON (Murphy et al., 2000). Zhu and Carreiro (2005) hypothesized that the recalcitrant nature of the organic N in soil systems coupled with its lack of seasonal variation with respect to plant N uptake meant larger contributions to leaching losses, hence a more environmentally important stature on organic N relative to inorganic N.

Plant residues are the primary sources of substrate for soil microorganisms. Plant residues contain substantial amounts of free amino acids, organic acids and sugars that are easily degraded and utilized by many microbes (Sylvia et al., 2005). Plant root exudates and sloughed-off cells from living plants are also a source of nutrition for microbes, and microorganisms will colonize the rhizosphere in much larger populations than in the bulk soil. Fungi are the pioneer species of the decomposition process due to the unique array of extracellular enzymes produced, although some bacteria—*Bacillus* and *Clostridium*, for example--are quite efficient in degradative processes as well (Sylvia et al., 2005). Once organic polymers have been broken down into monomeric components, mainly by fungi, bacteria contribute more to the mineralization process.

Approximately 30% of the N released by the hydrolysis of soil organic matter is in the form of amino acids (Stevenson, 1982) and produces other organic compounds that may be soluble but resistant to microbial decay (Smolander et al., 1995). Kubat et al. (1999) and Marschner et al. (2003) found that additions of mineral fertilizers significantly decreased proteolytic bacteria populations in soils while organic fertilizers increased populations. Impacts on the microbial biomass populations may also have an effect on the pool size of SON and its transformations in agroecosystems.

The potential for organic N uptake by plants in agroecosystems is unclear at this time. Research in boreal (Nasholm et al., 1998), grasslands (Jones et al., 2004), alpine (Lipson et al., 1999), and arctic (Jones and Kielland, 2002) ecosystems indicate that plants in these environments have the ability to absorb amino acids as a source of N. Agricultural soils are inundated with fertilizers containing mineral N species, however, unlike natural ecosystems, and agricultural crops are known to preferentially absorb mineral N. Appel and Mengel (1990, 1992) found that rape plants had no effect on the size of the SON pool of sandy soils although maintaining similar pool size cannot be interpreted as a lack of N transformations or supply to plants. Some evidence suggests that maize roots do scavenge SON using amino acid-specific transporters in root cells (Jones and Darrah, 1994). Findings by Okamoto and Okada (2004) indicate maize prefers mineral N consumption even under low N conditions as maize growth correlated well with mineral N concentrations in soil. Sorghum and rice growth under low mineral N conditions indicated their ability to utilize organic N as a major pathway for uptake (Okamoto and Okada, 2005). Fischer et al. (2002) isolated 6 amino acid permease enzymes responsible for amino acid transport across the cellular membrane in

Arabidopsis, a member of the family Brassicaceae. Actual uptake of amino acids by *Arabidopsis* is postulated as dependent on pH and the amino acid concentration within the apoplasm of the plant (Fischer et al., 2002).

Jones et al. (2005a) acknowledges the ability of plants to uptake organic forms of N, but states that the ability to do so does not imply it is a major pathway for N uptake due to intense competition with soil microorganisms and the availability of mineral N species. In organic rich soils, however, amino acid uptake by plants may function as a major pathway for N capture (Jones et al., 2005b). Further speculation on organic N uptake by plants suggests its function is to recapture amino acids lost in root exudation (Jones et al., 2005a).

Christou et al. (2005) attempted to estimate the average DON concentrations in soil solutions from 0-15 cm depth at 94 sites with 7 contrasting agricultural land use types. The shallow sampling depth of the soil solution does not consider the effects of plant uptake, N transformations, or soil adsorption in subsoil layers as DON is carried deeper into the soil profile. Organic N species would typically be higher in A horizons due to higher organic matter (Woods, 1989; Soudi et al., 1990) and higher microbial biomass. Surface soil solution DON concentration is important for measuring N losses due to erosion processes, but does not accurately represent DON within the entire soil system since DON concentrations are also effected by subsoil processes.

With respect to the impacts of organic N on water quality, Findlay and Sinsabaugh (2003) investigated the N uptake potential for both mineral and organic N species by bacterial communities in biofilms located on gravel under streambeds (hyporheic zone). Their study found that the biofilms had a more pronounced response to amino acids than to equimolar NH_4^+ as measured by a decrease in oxygen concentration in perfusion cores without altering biofilm community structure. The results of Brookshire et al. (2005) agree with Findlay and Sinsabaugh (2003) that biotic DON uptake exceeds inorganic N as applied to N-limited headwater forest streams.

Glibert et al. (2004) examined the impact of organic N on algal blooms at 7 sampling stations in Florida Bay. Glibert concluded that the phytoplankton had the capacity to take up organic N although uptake potential differs by phytoplankton species. Dissolved organic N has been shown to positively impact the populations of dinoflagellates, pelagophytes, and cyanobacteria—several species of these groups contributing to harmful algal blooms (Paerl, 1988; Berg et al., 1997; LaRoche et al., 1997; Lomas et al., 2001; Glibert et al., 2001; Glibert et al., 2004).

CONCLUSIONS

Brassica cover crops are a relatively new cover crop to the Atlantic coastal plain region of Maryland and investigations into species establishment, sowing dates, moisture needs, and their affect on N cycling should be conducted. By comparing previous studies on Brassicas and rye, insight may be gained into the best growing conditions and expectations in terms of nutrient management since rye is a well-established and popular cover crop in Maryland.

Many nutrient studies do not consider deep N (>60 cm) when approaching management issues. Plant N uptake analyses frequently do not include root measurements either. Similarly, studies involving sampling of soil water should include frequent (or continuous) sampling throughout the fall, winter, and spring leaching season, and sampling at proper depths within the soil that will provide a better reflection of the ability of a crop to conserve N and reduce leaching.

Most of the literature on organic N is focused on forest ecosystems, although some studies have been conducted on agricultural land. Leaching losses from natural ecosystems tend to be dominated by DON (Qualls et al., 1991; Qualls et al., 2000; Smolander et al., 2001) while agricultural DON losses are considerable (Christou et al., 2005), but may not be the greatest proportion of losses (Siemens and Kaupenjohann, 2002; Murphy et al., 2000). Soil SON may be present in amounts as great as mineral N in agricultural soils (Jensen et al., 1997; Bhogal et al., 2000). Studies on marine and freshwater nutrient enrichment indicate that phytoplankton are capable of absorbing both mineral and organic N, placing emphasis on DON inputs from terrestrial sources. Organic N cycling and management has not been fully researched at this time and should be considered for future study.

Lack of data on Brassica performance in the Maryland Coastal Plain concerning N retention has made the following study necessary. Reductions of soil mineral N, especially deep N (>60 cm depth), are important to decrease potentially leachable N from movement beyond the rooting zone of crop species. Plant N uptake as an indicator of N conservation by crops in fall and spring (for those winterhardy plants), soil N in crop plots, and monitoring of soil porewater N within plots will be used to assess the capabilities of Brassicas to conserve N within the plant-soil system and determine any influences that the cover crops may have on mineral and organic N in soil and porewater. Brassicas will be compared to rye as a benchmark for N scavenging performance, but Brassicas may have benefits to farmers that rye does not, such as weed and nematode suppression, lower C:N ratio and less microbial immobilization of nutrients, and increased subsequent crop yields.

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Table 1. Reduction in soil nitrate-nitrogen by various brassica cover crops, rye, and ryegrass compared to no cover control in various studies.

				Soil NO_3-N (kg ha^{-1})	
Reference	**Crop**	**Soil Texture**	**Planting/Sampling Date**	**0-1 m**	**0-2.5 m**
Thorup-Kristensen 2001	control	sandy loam	early Aug/mid Nov	129	
	forage radish			15	
	rape			9	
	rye			24	
Weinert et al 2002†	rape	Quincy loamy sand	late Aug/early March	25	
	rye			21	
	control			85	
	mustard			42	
Isse et al 1999	forage radish	clay	early Sept/late Oct	69.1	
	canola			70.1	
	ryegrass			73.4	
	control			136	
Kristensen and Thorup-Kristensen 2001	forage radish	sandy loam	early Aug/early Nov	11.9	18.1
	rye			32.2	59.4
	ryegrass			47.8	86.9
Vos and Van der Putten 2001‡	rye	sandy soil	mid Aug/mid March	47	
	rape			49	
	control			51	

† soil data from 60-120 cm depth in soil

‡ soil data from 0-90 cm depth in soil

Table 2. Biomass and N uptake measurements of various fall-harvested brassicas, rye and ryegrass across several studies

Reference	Crop	Shoot Biomass (kg/ha)	Root Biomass (kg/ha)	Shoot:Root	Total Recovered Biomass (kg/ha)	Root Depth (m)	Shoot Plant N Uptake (kg N/ha)	Root N Uptake (kg N/ha)	Whole Plant N Uptake (kg N/ha)
Thorup-Kristensen 2001[†]	Forage radish	4700	900	5.2	5600				160
	Rape	4000	1400	2.9	5400				148
	Rye	2100	1000	2.1	3100				91
Weinert et al 2002[‡]	Rape	3000					110		
	Rye	4600					133		
	Mustard	2900					62		
Isse et al 1999 [‡]	Forage radish	2810	336	8.4	3146		94	11.8	105.8
	canola	1960	146	13	2106		71	4.1	75.1
	Ryegrass	1460	103	14	1563		47	1.3	48.3
Isse et al 1999 [‡]	Forage radish	3120	522	6,0	3642		90	12.9	102.9
	canola	1920	87	22	2007		67	1.7	68.7
	Ryegrass	248	1691	0.15	1939		44	9.7	53.7
Kristensen and Thorup-Kristensen 2004	Forage radish	3960				2.4	157.6		
	Rye	2130				1.1	90.5		
	Ryegrass	4290				0.6	127.4		
Jackson et al 1993[§]	Oilseed radish	4128	2867	1.4	6995				200
	White mustard	5913	2273	2.6	8186				205
	Rye	4410	950	4.6	5360				129
Vos and Van der Putten 2001	Rye								153
	Rape								140
	Oilseed Radish								170
	Rye								123
	rape								138
Nielsen, 1997	B. napus cv.' Westar'					1.65			
Merrill et al, 2000	B. rapa cv. 'Reward'					1.14			
Black et al, 1991	Rape (unknown cv.)					1.5			
Lucas , 2004[¶]	Rye	2845	923	3.1	3768				
	Rye	4212	1388	3.0	5600				
	Rye	7537	2847	2.6	10384				
	Rye	3802	1578	2.4	5380				

[†] roots sampled to a depth of 1 cm ; [‡] roots sampled to unknown depth ; [§] roots sampled to 60 cm; [¶] roots sampled to 10 cm

CHAPTER III

BRASSICA COVER CROPS FOR MINERAL N RETENTION IN THE MARYLAND COASTAL PLAIN

ABSTRACT

Brassica cover crops are new to the mid-Atlantic region and N uptake capabilities have not been thoroughly investigated for effective N conservation. Forage radish (*Raphanus sativus* L. cv. Daichon), oilseed radish (*Raphanus sativus* L. cv. Adagio), and rape (*Brassica napus* L. cv. Dwarf Essex) were compared to rye (*Secale cereale* L. cv. Wheeler), a popular cover crop in the region, in N uptake ability and potential to decrease leaching N losses at 2 sites in Maryland, Beltsville and Wye. Plants were harvested in fall and spring for dry matter and N analysis. Soil samples from 105 to 180 cm depth were obtained in fall and spring for NH_4-N and NO_3-N analyses. Tension lysimeters were installed to depths of 75-120 cm to monitor soil porewater NO_3-N. Compared to rye, the Brassica shoots took up similar or greater amounts of N and caused similar decreases in soil NO_3^--N in fall and spring throughout the sampled profile compared to a weedy fallow control. Cover crops had no effect on soil NH_4-N. During the spring on coarse textured soil, porewater NO_3-N concentrations in winterkilled Brassica plots were greater than in control and overwintering Brassica and rye plots. On fine textured soil, all cover crops provided a similar decrease in NO_3-N concentration compared to the control. On coarse textured soils, it is suggested that the winterkilled Brassica cover crops be followed by an early-planted spring main crop.

INTRODUCTION

Agricultural N losses comprised 39% of the 159 million kilograms of N added to the Chesapeake Bay in 2003 (Chesapeake Bay Program, 2005). Nitrogen inputs may aggravate eutrophication of surface waters and increase drinking water contamination. The Chesapeake Bay is especially sensitive to nutrient pollution due to the large land to water ratio within the watershed (Pionke et al., 2000). Cover crops are an established means of reducing nutrient losses from agricultural lands (Jackson et al., 1993; Vos and Van der Putten, 2004; Weinert et al., 2002; Meisinger et al., 1991). The Maryland Winter Cover Crop Program provides financial assistance to farmers who choose to grow small grain cover crops such as rye (*Secale cereale* L.), wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), or oats (*Avena sativa* L.). Rape (*Brassica napus* L.) was added to the Winter Cover Crop Program in 2005 based on research conducted at the University of Maryland.

Rye is a popular cover crop in the Atlantic Coastal Plain and its N scavenging capacity and adaptability to the soils and climates in the region has been well documented (Shipley et al., 1992; Staver and Brinsfield, 1998; Coale et al., 2001). Rye was therefore used in the present study as a benchmark to which Brassica performance was compared.. Brassica cover crops such as forage radish (*Raphanus sativus* L.), oilseed radish (*Raphanus sativus* L.), and rape are relative newcomers to the Maryland Coastal Plain, but have proved successful at capturing N in Europe, Canada, and the western regions of the United States, in some cases outperforming rye in plant N uptake and decreasing soil NO_3-N (Jackson et al., 1993; Laine et al., 1993; Thorup-Kristensen, 1994; Isse et al., 1999; Thorup-Kristensen, 2001; Weinert et al., 2002; Kristensen and Thorup-Kristensen, 2004; Vos and Van der Putten, 2004). Kristensen and Thorup-Kristensen (2004) observed total plant N uptake of forage radish to be significantly greater than rye with values of 158 and 90.5 kg N ha^{-1} respectively. Response to soil N by the Brassicas declined much more rapidly with delay in planting during the fall than with rye (Vos and Van der Putten, 1997), and Thorup-Kristensen (2001) suggests that Brassicas are likely to outperform rye only during longer growing seasons.

Dissolved nitrate-N moves through the soil by mass flow and may quickly be transported beyond the rooting zone of plants. For most effective N conservation, cover crops need extensive and deep rooting systems that grow deep quickly (Meisinger et al., 1991; Sainju and Singh, 1997). Nitrogen uptake from deep soil layers has a greater effect on decreasing leaching losses than N uptake from surface layers (Thorup-Kristensen and Nielsen, 1998; Thorup-Kristensen, 2001). Kristensen and Thorup-Kristensen (2004) measured ^{15}N uptake of 6.2 and 0.1 mg N $subplot^{-1}$ (0.9 m x 0.8 m subplot) by forage radish and rye respectively at 1.1 m soil depth. Soil profile NO_3-N in the top 1.0 m was 11.9 and 32.2 kg ha^{-1} for forage radish and rye respectively, and soil profile NO_3-N from 1.0-2.5 m depth was 6.2 and 27.2 kg ha^{-1} for forage radish and rye respectively, clearly indicating the effectiveness of forage radish to capture N from deep soil layers (Kristensen and Thorup-Kristensen, 2004). Vos and Van der Putten (1998) concluded that the cereal rye rooting systems did not behave differently from those of the rape and oilseed radish root systems to a depth of 40 cm based on root length per unit area and aboveground biomass.

This study compares forage radish, oilseed radish, and rapeseed, to rye in relation to plant N uptake, depletion of soil profile NO_3-N, and maintenance of low soil porewater

NO_3-N. Brassicas were also compared to weedy fallow control plots to quantify soil profile NO_3-N depletion and decreases in soil porewater NO_3-N.

MATERIALS AND METHODS

Field experiments to study N capture by several cover crops were conducted at two sites in Maryland [University of Maryland Central Maryland Research and Education Center at Beltsville facility (CMREC) and Wye Research and Education Center (WREC)] from August 2003 through May 2005. The experimental design at both sites was a randomized complete block with 4 replications.

CMREC Site Description

The soil at CMREC was a complex of Cedartown and Evesboro loamy sands, averaging 17 mg organic matter g^{-1} soil and pH 6.5. In two blocks the dominant soil was Evesboro loamy sand (mesic, coated Lamellic Quartzipsamment) with a surface horizon texture of 78% sand and 6% clay. The clay content is less than 6% from 10 cm to 120 cm depth. In the other two blocks the dominant soil was Cedartown loamy sand (siliceous, mesic Psammentic Hapludult), with a surface texture of 82% sand and 7% clay. The clay content increases to 15-20% in the argillic horizon (80-120 cm). The Cedartown loamy sand shows evidence of a seasonal perched water table from 90 cm to 120 cm depth during the winter and early spring. CMREC is situated in an area with moist continental climate (39.02° N, 76.53° W; thirty-year average of 1112 mm mean annual precipitation and 12.8 ºC mean annual temperature). Figure 1.1 shows the daily precipitation and average daily temperature for CMREC over the course of the experiment.

CMREC Field Management

The soil at CMREC was last plowed March 31, 1999, and was then put in a no-till corn (*Zea mays* L.) / winter wheat (*Triticum aestivum* L.)/ double crop soybean (*Glycine max* (L.) Merr.), rotation until August 2003. During this experiment a corn–full season soybean rotation was maintained.

This experiment used plots and treatments within a larger experiment that had plots for both drilled and broadcast cover crop plots. Plots used during fall 2003-spring 2004 (CMREC1) for cover crop treatments were not used for the second year of the experiment in fall 2004-spring 2005 (CMREC2). The plots used during the second year were embedded within the larger experiment, but had a failed stand (zero biomass) of broadcast cover crops during fall 2003-spring 2004. Neither CMREC1 nor CMREC2 had prior cover crop rotations although both sets of plots were subjected to the same main crops during the study.

<u>CMREC Year 1 (CMREC1)</u>

At CMREC1 on August 13, 2003, forage radish (*Raphanus sativus* L. cv. Daichon) and oilseed radish (*Raphanus sativus* L. cv. Adagio) were no-till drilled into wheat stubble using a no-till drill at 13 kg ha^{-1} seeding rate and fertilized with 56 kg ha^{-1} of N as $(NH_4)_2SO_4$ to ensure adequate cover crop nutrition. Cover crop plots were 3.7 m by 9.1 m with 15 cm row spacing. All cover crops winterkilled during December 2003-January 2004. The plots at CMREC1 and CMREC2 were planted to glyphosate-resistant soybeans (cultivar NK S39-Q4; 155,555 seeds ha^{-1}; 38 cm row spacing) on May 12, 2004. Soybeans were mowed at reproductive stage R8 in the CMREC2 plots on August

18, 2004 to provide a source of mineralizing organic N (200 kg total N ha^{-1}, as determined by biomass and N analysis) for evaluating the N uptake performance of cover crops planted later in the month.

<u>CMREC Year 2 (CMREC2)</u>

On August 25, 2004, forage radish, oilseed radish, rape (*Brassica napus* L. cv. Dwarf Essex), and rye (*Secale cereale* L. cv. Wheeler) were planted at CMREC2 (using 13, 13, 10 and 126 kg seed ha^{-1}, respectively). Cover crop plots were 3.7 m by 9.1 m with 15 cm row spacing. Forage and oilseed radishes winterkilled in December 2004-January 2005, and rape and rye were killed using glyphosate (2.3 L ha^{-1} AI; N-(phosphonomethyl)-glycine) on April 27, 2005. On May 5, 2005, lime was surface applied at a rate of 1120 kg ha^{-1}. The CMREC2 plots were planted to corn (Pioneer 34B62; 65,000 seeds ha^{-1}; 76 cm row spacing) on May 10, 2005.

WREC Site Description

The soil at WREC is a Mattapex silt loam (fine-silty, mixed, active, mesic Aquic Hapludult). The Ap horizon is characterized by 27% sand, 18% clay, 20 mg organic matter g^{-1} soil and pH 6.3. Mattapex silt loam was formed from loess deposits overlaying coastal plain sediments. An argillic horizon (26% clay) was observed from 30-50 cm depth and conversion to loam-loamy sand coastal plain sediments occurs between 100-120 cm depth. WREC (39.03° N, 76.04° W) has a moist continental climate with mean annual precipitation of 1103 mm and mean annual temperature of 13.3°C based on a 30 year average (Figure 1.1).

WREC Field Management

WREC was in no-till management for this experiment and the previous 5 years with a corn and soybean rotation. Cover crop plots were 3.2 m by 9.1 m with 15 cm row spacing. Plots used from fall 2003-spring 2004 (WREC1) had been planted to forage radish, oilseed radish, rape, and rye cover crops since August 2001. Plots used during fall 2004-spring 2005 (WREC2) had no history of cover cropping since 1983 (no information available prior to 1983). The plots at WREC2 were adjacent to WREC1 and no differences in profile characteristics were observed between the two locations. Treatment plot dimensions were 3.2 m by 9.1 m with 15 cm row spacing.

<u>WREC Year 1 (WREC1)</u>

After applying glyphosate (0.93 L ha^{-1} AI) on August 1, 2003, forage radish and oilseed radish, rye, and rape (seeding rates, 14, 14, 126, 10 kg ha^{-1}) were planted at WREC1 on August 19, 2003. Forage and oilseed radishes winterkilled during December 2003-January 2004 and the remaining cover crops were killed by rolling on May 5, 2004, and sprayed with glyphosate (1.4 L ha^{-1} AI) the following day. The plots were planted to soybeans (cultivar NK S39-Q4; 155,000 seeds ha^{-1}) with 76 cm row spacing on May 21, 2004, using a John Deere 7200 planter and Kinze Brush seed meters. Glyphosate (1.9 L ha^{-1} AI) was sprayed for weed control within soybeans on June 23, 2004. Soybeans at WREC2 were mowed at reproductive stage R8 on September 18, 2004, to provide a mineralizing organic source of N (250 kg N ha^{-1}) for evaluating the N capture capacity of the cover crops during fall 2004-spring 2005. Soybeans were harvested and analyzed for shoot tissue N to determine the quantity of mineralizing organic N provided for the following cover crops.

WREC Year 2 (WREC2)

Forage radish, rye, and rape (seeding rates 14, 126, and 10 kg ha^{-1}) were planted at WREC2 on September 24, 2004. The cover crops that did not winterkill, rape and rye, were sprayed with glyphosate (1.9 L ha^{-1} AI) on May 3, 2005, and corn (Pioneer 34B62; 40,000 seeds ha^{-1}) was planted on May 19, 2005, without N fertilizer. The crop was side dressed with N fertilizer in mid-June, after the last sample in this study were collected.

Field sampling

Three 0.25 m^2 quadrats of plant samples were harvested in each plot and the dry weight of the entire quadrat was measured and subsampled for nutrient analyses in fall 2003-spring 2004. In fall 2004-spring 2005, two 0.25 m^2 quadrats of plant tissues were sampled from each plot and the fresh weight of the harvested quadrat was measured then subsampled for analysis of water and nitrogen contents. Plant shoots were harvested for all treatments, but roots were collected only for the Brassicas, which have large fleshy roots, with about a third protruding above ground. The main fleshy roots were sampled to approximately 15 cm depth by pulling up the plant, then washing the roots free of soil. Plant biomass measurements (Equation 1) were used in conjunction with N analysis to determine plant N uptake using the following equation:

(1) $$\text{N uptake (kg ha}^{-1}\text{)} = \text{plant biomass (kg ha}^{-1}\text{)} \times \text{N content (kg/kg)}$$

The locations of the plant and soil samples were randomly chosen at least 1 m from the edge of the plot. Plants were sampled by block over several days in early October and

November, and April through May of each year. After harvest, plants were dried at 65° C and ground to pass a 1 mm sieve.

Soil was sampled to depths of 105-180 cm depending on site conditions using a Veihmeyer corer (Devera et al, 1980; Veihmeyer, 1929), placed in 4 cm diameter PVC troughs for examination, and divided into 15 cm increments. Three soil cores were taken in each plot and homogenized to represent the plot. Soil samples were taken on the same dates as plant samples. Soil bulk density was calculated for each 15 cm increment to estimate kg ha^{-1} of soil N:

(2) $$\text{Bulk density (g cm}^{-3}\text{)} = \frac{M}{\pi r^2 l}$$

where M = dry mass of sample in g, r = radius of the cutting tip, and l = length of the sample (15 cm). Bulk density for soil samples greater than 30 cm in depth were averaged over the sampling period for each location in 30 cm increments (30-60, 60-90, 90-120, 120-150, 150-180 cm). Bulk density for CMREC was averaged over two years while bulk density was averaged for WREC1 and WREC2 separately due to differences in plot locations between study years. Surface soil samples (0-15, 15-30 cm) were not averaged over years, and the bulk density for the individual sampling date was used for calculations due to the mutability of surface horizons by freeze-thaw cycles and physical disturbances.

Tension lysimeters (1.9 cm inner diameter) were installed in plots at both sites in February-March 2004 (1 per plot) and February-March 2005 (2 per plot) to monitor pore water NO_3^--N concentration and were sampled weekly until May of each year when they were removed for the planting of the summer crop. Lysimeters were installed to 75 cm depth at WREC1 and 90 cm at CMREC1 during February 2004. In February 2005, lysimeters were installed to 90 cm depth at WREC2 and 120 cm at CMREC2. An 80 to 90-kPa vacuum was applied to the lysimeter with a hand pump and porewater collected over a 24 to 48 hour period.

The mass of N lost by leaching was approximated as follows (Equation 3):

(3) N loss = soil porewater NO_3-N concentration x leaching water volume

where the water volume was estimated as precipitation – evapotranspiration. This simplification assumes that there was no surface runoff and that soil storage was neglible because the soils remained near field capacity throughout the leaching season. Evapotranspiration was estimated from average monthly temperature using the Thornthwaite method (Dunne and Leopold, 1978), (Equation 4).

(4) $$E_t = 1.6\left[\frac{10Ta}{I}\right]^a$$

Where E_t = potential evapotranspiration (cm mo^{-1})

Ta = mean monthly air temperature (º C)

I = annual heat index = $\sum_{i=1}^{12}\left[\frac{T_{ai}}{5}\right]^{1.5}$

`

$a = 0.49 + 0.01791 - 0.0000771I^2 + 0.000000675I^3$

A correction factor of 0.99 for March and 1.10 for April were multiplied by the calculated potential evapotranspiration given by the Thornthwaite method to adjust for latitude (sites located at 39ºN latitude; correction factor for 40ºN latitude used). Precipitation data was used to calculate percolating water volume assuming no surface runoff, and evapotranspiration was subtracted from percolating water volume to determine the volume of Leaching volume was multiplied by the monthly average concentration of NO_3-N in soil porewater to obtain an approximate mass per area leaching loss amount (kg ha^{-1}).

Laboratory analyses

Nitrogen analyses were performed on plant, soil, and pore water samples. Nitrate-N and NH_4-N were extracted from soil using 0.5 *M* K_2SO_4 (1:10 dilution). A copperized cadmium reduction column in a flow-injection autoanalyzer (Technicon Autoanalyzer II; Technicon Industrial Systems, Tarrytown, NY) was used for NO_3-N analysis (Technicon method no. 487-77a) of soils. Ammonium-N was determined using an ammonia gas selective electrode (Orion EA940; Thermo Electron, Waltham, MA) after addition of ionic strength adjusting solution and raising pH to 13 (Banwart et al, 1972). Plant total nitrogen (TN) was analyzed using a combustion analyzer (CHN 2000; LECO, St. Joseph, MI) for samples (ground to pass 1 mm sieve) collected during August 2003-May 2004. For samples collected during August 2004-May 2005, plant TN was measured using an inductively coupled plasma (ICP) spectrometer. Soil pore water was analyzed for NO_3-N colorimetrically (Cataldo et al., 1975).

Statistical analysis

Statistical analysis of data was conducted to compare each 15 cm increment of soil sampled, for the sum of all N in the sampled (0-105+ cm) profile, for plant dry matter, for plant tissue N concentration, and for plant N uptake using ANOVA as calculated by SAS Proc Mixed (SAS Institute, Cary, NC). Block effects were treated as random variables while cover crop treatments were considered fixed effects. Pore water samples were analyzed using repeated measures ANOVA (time as the repeated variable) with SAS Proc Mixed for the average porewater N over the sampling season. Analysis of variance was also performed to compare treatments for each date sampled. Tukey Multiple Mean Comparison Tests and orthogonal contrasts were performed to compare cover crops to one another and to the control. Due to limitations on the number of soil subsamples obtainable per plot (3 cores per plot), a P level < 0.10 was considered significant.

RESULTS

Cover Crop Dry Matter

Cover crop dry matter varied among sites and species during fall of 2003 and 2004 (Table 1.1). Brassicas had similar fall shoot dry matter production at each site for each fall season although root (fleshy part only sampled) dry matter differed between sites with radishes producing significantly more root dry matter than rape. A portion of the root biomass of the radishes might be considered aboveground material because the thickest part of the root actually grew above ground and frost heaving forced more root tissue to the soil surface. No root comparisons were made between rye and the Brassicas. Rye shoot dry matter was significantly lower than the Brassicas at CMREC2 and WREC2 in fall. Forage radish had the highest root dry matter production of the Brassicas in fall for CMREC2 and WREC2. Oilseed radish had the highest shoot biomass production at CMREC1 and CMREC2 in fall.

Rape and rye shoot dry matter were not significantly different in spring at any site or year. Rape and rye shoot dry matter ranged from 3100-4700 kg ha^{-1} at CMREC2, and 2000-2100 and 6200-6500 kg ha^{-1} for WREC1 and WREC2 in spring. Rape root dry matter was 1532 and 2841 kg ha^{-1} for CMREC2 and WREC2, respectively. Rape root dry matter was not collected at WREC1 in spring.

Cover Crop N Uptake

Within each site in fall, the percentage of N in the Brassica and rye shoot tissues did not differ (Table 1.2). However, following soybeans in fall 2004, the Brassica and rye tissue N was twofold higher than in 2003. Carbon to N ratios increased in spring with rape shoots (16-18 mg N g^{-1} tissue at WREC1 and WREC2, 26 mg g^{-1} at CMREC2) containing greater N than rye shoots (14-16 mg g^{-1}). Rape roots at CMREC2 (14 mg N g^{-1}) captured twice the N as those at WREC2 (7 mg g^{-1}) in spring 2005.

Nitrogen uptake by the cover crops paralleled biomass production (Table 1.3). Brassica N uptake by aboveground tissues increased from fall 2003 to fall 2004 following soybeans. Brassica shoot N uptake at CMREC2 and WREC2 was significantly greater than rye shoots ($p<0.03$). During fall 2004, forage radish roots captured almost twice as much N as oilseed radish at CMREC2 and around three times the N taken up by rape at both CMREC2 and WREC2. Rape and rye shoot N uptake in spring were similar at WREC1 (30-38 kg N ha^{-1}), WREC2 (83-117 kg N ha^{-1}), and CMREC2 (73-84 kg N ha^{-1}). Rape root tissue captured 21.4 kg N ha^{-1} at CMREC2 and 19.6 kg N ha^{-1} at WREC2 in spring.

Cover Crop Influence on Soil Mineral N

Cover crops had no effect on soil NH_4-N during the course of the experiment with the exception of fall at WREC2 when rye plots had 77.4 kg ha^{-1} NH_4-N compared to the Brassica and control plots (86.4-94.8 kg ha^{-1}) (data not shown). Seasonal and fertilizer effects were evident on the soil NH_4-N content. Ammonium-N in the soil profile at CMREC1 and WREC1 decreased from fall to spring, but increased from fall to spring at CMREC2 and WREC2 (following the mowed soybeans).

Cover crop treatments significantly reduced soil NO_3-N in both fall and spring (Figures 1.2, 1.3) compared to no cover control plots. Brassicas and rye decreased soil profile NO_3-N (0-150 cm) at CMREC in fall compared to control plots, paralleling results at WREC1 (0-120 cm) and WREC2 (0-105 cm) in fall with decreases in several depth increments to 1.0 m depth. By spring, forage and oilseed radish plots had increased the soil profile NO_3-N (0-60 cm) while rape and rye continued to decrease soil profile NO_3-N by plant uptake compared to control plots.

Porewater Nitrate

In spring 2004, NO_3-N concentrations in soil porewater samples were very low (< 2 mg/L) and were unaffected by crop treatments at either site in spring 2004 (Figure 1.4). Calculated leaching losses for March 2004 (2.22 kg NO_3-N ha^{-1} mo^{-1}) were less than those for April 2004 (5.01 kg NO_3-N ha^{-1} mo^{-1}) at CMREC1. WREC1 had greater leaching losses in April (3.12 kg ha^{-1} mo^{-1}) than during March (2.90 kg ha^{-1} mo^{-1}). Nitrate-N flushes corresponded with precipitation events at both sites.

Cover crops affected porewater NO_3-N in spring 2005 (Figure 1.4). Forage and oilseed radish plots had comparable if not greater amounts of NO_3-N in porewater as control plots while rape and rye plots showed marked decreases. Control plots had significantly greater concentrations of NO_3-N in soil porewater (5.0-7.0 mg L^{-1}, 58 kg ha^{-1} mo^{-1}) than did the forage radish (4.1-5.2 mg L^{-1}, 42 kg ha^{-1} mo^{-1}), and rape and rye cover crops (0.06-0.25 mg L^{-1}, 1.9-6.1 kg ha^{-1} mo^{-1}) during March 2005 at CMREC2. During April 2005, the porewater from the forage radish plots (ranged from 10-17 mg NO_3-N L^{-1}, 104 kg NO_3-N ha^{-1} mo^{-1}) at CMREC2 contained as much as five times the NO_3-N concentration as porewater from the control plots (2.4-2.6 mg L^{-1}, 19 kg ha^{-1} mo^{-1}). Rape and rye plots, respectively, at CMREC2 had the lowest NO_3-N leaching losses (March: 0.06-0.25 mg L^{-1}, 1.9-6.1 kg ha^{-1} mo^{-1}; April: 0.04-0.20 mg L^{-1}, 0.2-0.27 kg ha^{-1} mo^{-1}) in spring 2005. Rape and rye plots (0.29-0.34 kg NO_3-N ha^{-1} mo^{-1}) had markedly lower leaching losses in April 2005 at WREC2 compared to control plots (42 kg ha^{-1} mo^{-1}) although forage radish losses (6.0 kg ha^{-1} mo^{-1}) were not significantly different than control.

Discussion

Brassicas perform similarly if not better than rye in their ability to capture NO_3^--N. Shoot biomass and N uptake by the Brassicas was greater than that of rye. Brassica roots also have substantial N storage capacity as measured in fall (Table 1.3). Similar percentages of N were measured within the shoot tissues of both Brassicas and rye (also noted by Holderbaum et al., 1990; Wagger and Mengel, 1988), and with similar measured soil NO_3^--N depletions in the cover crop plots, it may be assumed that the rye roots captured at least as much N as did Brassica roots. Rape roots (fleshy parts only, ~10-15 cm depth) ranged between 22-30% of total dry matter in this experiment, which is comparable to rye with 24-29% of total dry matter as roots to 10 cm depth (Lucas 2004). Forage and oilseed radishes were much more variable in dry matter partitioning with 23-62% of total dry matter as roots.

While the fractions of N within the crop tissues did not differ at each site and season, the cover crops that followed mowed soybeans at CMREC2 and WREC2 had significantly higher fractions of N in shoots and roots than did those crops at CMREC1 and WREC1. Plant shoot and root N uptake and root biomass by crops at CMREC2 and WREC2 was also greater than CMREC1 and WREC1. Shoot biomass between study years was not affected.

Fall soil NO_3^--N in the Brassica and rye plots at several depth increments throughout the profile sampled was significantly less than that of control plots. During the spring, rape and rye plots had significantly less NO_3^--N than the control and radish plots. Forage and oilseed radish released N in late March-April after winterkilling, resulting in greater concentrations of NO_3-N in both the soil profile and in porewater than the rape and rye. Weinert et al. (2002) noted that overwintering cover crops were typically more effective at soil NO_3-N depletion than species that winterkill.

Forage and oilseed radishes significantly decreased soil NO_3-N in the profile at CMREC1 during the fall (Figure 1.2), and spring NO_3-N and mineral N measurements indicate very little mineralization of plant biomass N from fall to spring compared to the control plots (Table 1.4). The apparent lack of mineralization of the decaying radish tissues is also supported by soil porewater data (Figure 1.4) and calculated leaching losses. The deeply rooted radishes captured N throughout the soil profile, storing it in shoots and the surface portion of the taproot. After cold weather killed the radishes in late December-early January, the N stored in the shoots and taproot was released into the surface soil, mineralized, and available for the following crop to use. Temperatures during the time period went below freezing only during January 2004 (Figure 1.1) and it is likely that decomposition of the radishes began during late January and early to mid-February 2004. Magid et al. (2004) noted that decomposition of forage radish and rye occurred at temperatures as low as 3°C with no N immobilization by microbial biomass until 9°C thereby allowing a slow leak of unimpeded N to be potentially lost. Temperatures at CMREC1 did not reach 9°C until late March-early April, and coupled with low precipitation during March, allowed for little N movement in the soil profile and possible immobilization by microorganisms if the site was N-limited. Radish tissues harvested in October at CMREC1 held approximately half the N as did radish tissues from CMREC2 ($p<0.10$) in fall which had a large supply of potentially mineralizable N released by the mowed soybeans, supporting the probability that CMREC1 may have been N-limited. Thorup-Kristensen (1993) noted decreased N uptake by cover crops in soils with lower N reserves compared to soils with higher soil N, but N uptake paralleled the decreased biomass production of the crops without change in the C:N ratios except for Italian ryegrass (*Lolium multiflorum*). The 143 to 157 kg ha^{-1} reductions of mineral N in the soil profile of the radish plots compared to the control plots from fall to spring at CMREC1 are very similar to the total plant N uptake in the fall (127 to 143 kg ha^{-1}) (Table 1.4).

Brassica and rye cover crop plots conserved significantly more soil NO_3^--N compared to control plots at CMREC2 during November 2004-April 2005 (Table 1.5). The preceding soybeans killed in fall 2004 provided 200 kg ha^{-1} of potentially mineralizable organic N. A large flush of NO_3-N was observed in soil porewater from the forage radish plots during late March-early April as compared to the control, rape, and rye plots (Figure 1.4) as precipitation flushed the N released from tissue decomposition deeper into the soil. Radishes were able to take up more N in fall than rape and rye, depleting the soil profile of NO_3-N more efficiently, and deposited greater amounts of NO_3-N on the soil surface during the spring where it could be available for the following crops. Rape and rye continued to capture N throughout the spring with relatively little release of N back into the soil. The sum of plant N uptake and estimated NO_3-N leaching losses in spring at CMREC2 accounted for >80% of the difference in soil profile NO_3-N between November 2004 and April 2005. Negligible amounts of porewater NH_4-N were measured as consistent with Weil et al. (1990). Water quality concerns remain for the addition of forage and oilseed radish into the crop rotation on coarse textured soils due to the elevated concentrations of NO_3-N at CMREC2 during April 2005 (>10 mg L^{-1}).

Changes in soil NO_3^--N and mineral N at WREC2 (Table 1.6) differed from those of CMREC2. Finer textured soils like that at WREC are able to retain a greater amount of N than coarse soils like those at CMREC as also observed by Beaudoin et al. (2005) in France, and elevated NO_3-N leaching losses in porewater were not prevalent for the forage radish plots. Forage radish plots had similar soil NO_3-N amounts in the soil profile from fall to spring at WREC2 (Table 1.6) as the N captured from the deeper portion of the soil profile was redeposited at the surface through the decomposition of shoot and taproot tissues (Figure 1.3). Approximately 60-68% of the total plant N uptake of radishes was returned in mineral form to the soil at CMREC2 by the sampling dates in spring, but mineral N increases from fall to spring at WREC2 were only 5% of the forage radish uptake. Rape and rye continued to deplete soil NO_3-N at WREC2 during the spring resulting in significant reductions for both fall and spring. Only 2 blocks at WREC1 were sampled for plants and soil in fall 2003 therefore a N balance was not calculated due to lack of sufficiently replicated information.

Brassica cover crops and rye demonstrated the potential to decrease the quantities of soil NO_3^--N lost from plant-soil ecosystems by leaching as compared to winter fallow control plots, but certain species may be suitable for only certain sites and crop rotations. Depletions in soil NO_3-N were observed throughout the profile in fall at both CMREC and WREC. The release of N from decomposing forage and oilseed radishes tissues in spring may cause high nitrate-N concentration in porewater, depending on soil texture (Figure 1.4). In sandy soils, porewater N concentrations may be greater than 10 mg NO_3-N L^{-1} as biological activity increases with warmer weather and precipitation washes N deeper into the soil. Therefore, an early-planted, N-demanding crop such as corn or potato should be used following the winterkilled radish cover crop on course textured soils. Finer textured soils are less subject to leaching due to lower percolation, increased ability to protect organic matter from microbial decay (Kristensen et al., 2000), and higher cation exchange capacity compared to sandy soils. Rape and rye continue to capture soil N into the spring since it does not winterkill as do the radishes, and would be appropriate to use on both coarse and fine textured soils with main crops planted later in May or June.

CONCLUSIONS

The Brassica cover crops, forage radish, oilseed radish, and rape, performed similarly if not better than rye in the conservation of soil mineral N in fall. All cover crops decreased soil mineral N losses compared to winter weed control plots. Plant N uptake by the Brassicas was greater than or equal to rye during fall, and rape N uptake matched rye uptake during spring. In spring, the radish plots had larger losses of NO_3-N through leaching on coarse-textured soil.

Cover crop additions to crop rotations should take into consideration the release of N in relation to the N demands of the main crop, as well as the impact of soil texture on the timing of potential leaching of NO_3-N both in fall and spring. Brassica crops that winterkill release N from plant tissues early in spring and may contribute large amounts of NO_3-N to leaching losses if a main crop is not planted early enough to recapture this N. Early planting of a subsequent summer crop will be especially important to minimize spring leaching losses in coarse textured, well to excessively drained soils. Brassica crops such as rape that do not winterkill continue to capture soil NO_3-N into the late spring. Nitrogen taken up by rape or rye cover crops would be more likely to benefit later planted rather than earlier planted summer crops because N release would occur later than with the winterkilled Brassicas.

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Figure 1.1. Daily precipitation and daily average temperature at University of Maryland Central Maryland Research and Education Center-Beltsville facility (CMREC) and Wye Research and Education Center (WREC) from August 1, 2003 through May 31, 2005.

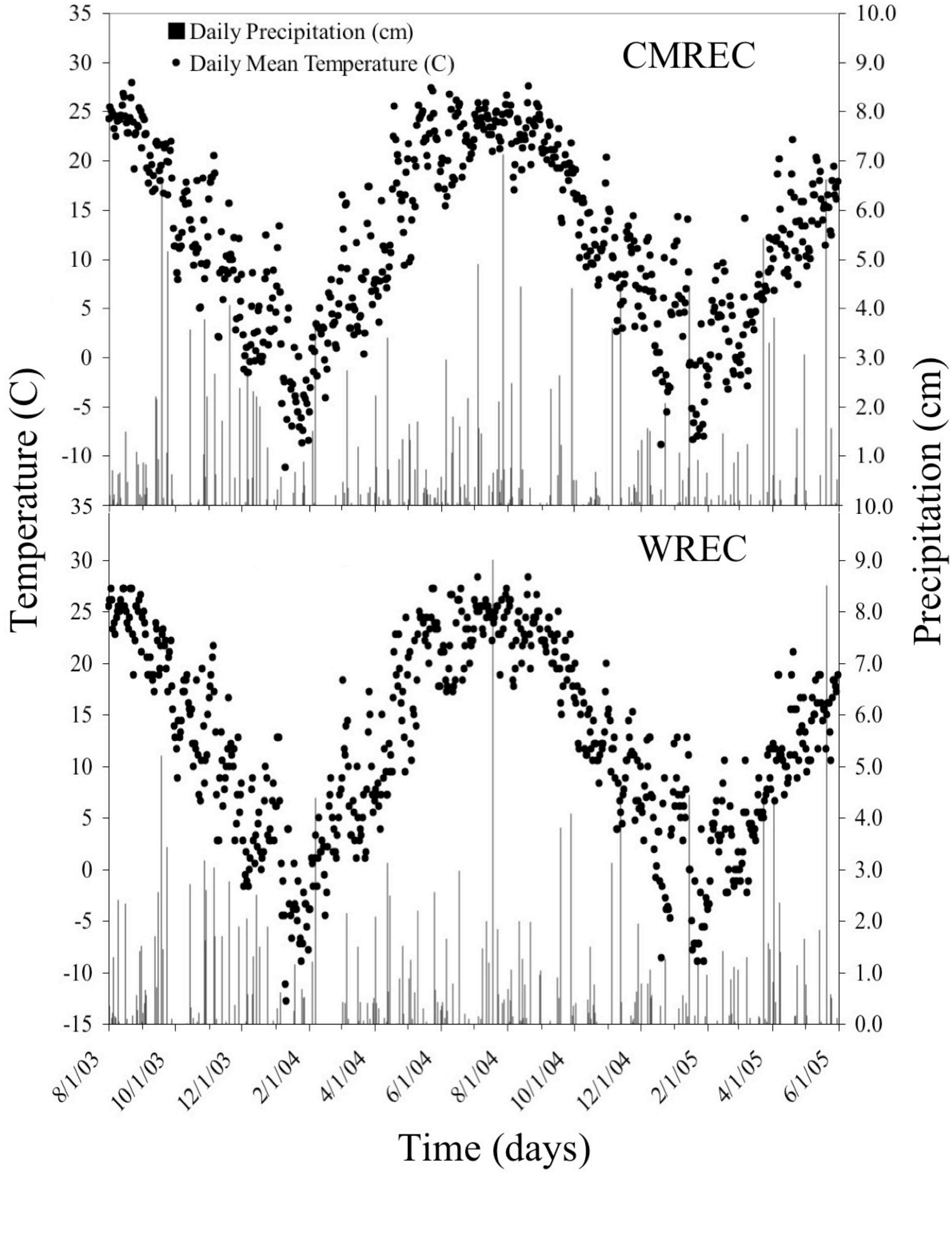

Table 1.1. Plant dry matter sampled near maximum growth in fall 2003 and 2004 at University of Maryland Central Maryland Research and Education Center-Beltsville facility (CMREC) and Wye Research and Education Center (WREC). Root samples were of the main portion of the Brassica taproot. Small letters indicate significantly different means for each site and year, $p<0.1$.

		2003					2004				
		CMREC1		WREC1‡			CMREC2			WREC2	
		kg ha^{-1}		kg ha^{-1}			kg ha^{-1}			kg ha^{-1}	
Cover Crop	df†	Shoot	Root	Shoot	Root	df†	Shoot	Root	df†	Shoot	Root
Forage radish		4650a	1443a	2433a	2920a		2432ab	3982a		4343ab	3843a
Oilseed radish		4912a	1580a	1993a	1580a		3848a	2172b			
Rape				2987a	1100a		3232a	1404b		5053a	1413b
Rye				1974a			1679b			3210b	
Block (Mean Square)	3,3	0	0			3,3	0	677088	3,3	0	171980
F value		0.150	0.550				16.1	8.42		6.78	48.2
P value		0.724	0.512				0.001	0.018		0.029	0.006
Error (Mean Square)	3,3	913506	67785			9,6	221092	832268	6,3	509546	245064
<u>Contrasts p-value</u>											
Brassicas v. rye							0.0004			0.0146	

†degrees of freedom are listed as shoots, roots

‡data for WREC1 represents only 2 blocks

Table 1.2. Plant tissue N (mg N g^{-1} tissue) sampled near maximum growth in fall 2003 and 2004 at University of Maryland Central Maryland Research and Education Center-Beltsville facility (CMREC) and Wye Research and Education Center (WREC). Root samples were of the main portion of the Brassica taproot. Small letters represent significantly different means within site and year, p<0.1.

		2003					2004				
		CMREC		WREC‡			CMREC			WREC	
		mg N g^{-1} tissue		mg N g^{-1} tissue			mg N g^{-1} tissue			mg N g^{-1} tissue	
Cover Crop	df†	Shoot	Root	Shoot	Root	df†	Shoot	Root	df†	Shoot	Root
Forage radish		21.4a	1.84a	18.6a	11.5b		39.3a	3.12a		36.0a	16.2a
Oilseed radish		20.0a	1.66a	18.8a	12.7a		39.3a	2.86a		-	-
Rape		-	-	16.8a	12.4ab		35.7a	2.45a		34.2a	15.1a
Rye		-	-	23.9a	-		39.4a	-		30.8a	-
Block (Mean Square)	3,3	5.6E-06	0			3,3	0	6.4E-06	3,3	0	3.0E-06
F value		0.20	0.42				0.45	1.15		0.51	0.3
P value		0.6844	0.564				0.7256	0.3783		0.6244	0.6213
Error (Mean Square)	3,3	1.2E-05	1.5E-05			9,6	3.0E-05	3.9E-05	6,3	5.3E-05	8.0E-06
<u>Contrasts p-value</u>											
Brassicas v. rye							0.7035			0.3787	

†degrees of freedom are listed as shoots, roots
‡data for WREC1 represents only 2 blocks

Table 1.3. Nitrogen uptake by plants sampled near maximum growth in fall 2003 and 2004 at University of Maryland Central Maryland Research and Education Center-Beltsville facility (CMREC) and Wye Research and Education Center (WREC). Root samples were of the main portion of the Brassica taproot. Small letter indicate significantly different means, p<0.1.

		2003					2004				
		CMREC1		WREC1[‡]			CMREC2			WREC2	
		kg N ha^{-1}		kg N ha^{-1}			kg N ha^{-1}			kg N ha^{-1}	
Cover Crop	df[†]	Shoot	Root	Shoot	Root	df[†]	Shoot	Root	df[†]	Shoot	Root
Forage radish		99.5a	27.3a	44.2a	33.5a		95.6bc	118a		155ab	60.6a
Oilseed radish		97.8a	26.5a	36.6a	19.8a		151a	63.0b			
Rape				49.6a	13.6a		118ab	33.5b		171a	21.2b
Rye				42.6a			65.6c			99.0b	
Block (Mean Square)	3,3	0	0			3,3	0	375	3,3	0	0
F value		0.01	0.02				7.09	11.4		4.51	35.4
P value		0.929	0.907				0.010	0.010		0.064	0.010
Error (Mean Square)	3,3	632	82.1			9,6	738	642	6,3	1258	87.8
<u>Contrast p-value</u>											
Brassicas v. rye							0.006			0.026	

[†]degrees of freedom are listed as shoots, roots
[‡]data for WREC1 represent only 2 block

Figure 1.2. Soil nitrate-nitrogen amounts for each 15 cm depth increment sampled at University of Maryland Central Maryland Research and Education Center-Beltsville facility (CMREC) from October 2003-April 2005. Small letters indicate significantly different means within a depth, p<0.1.

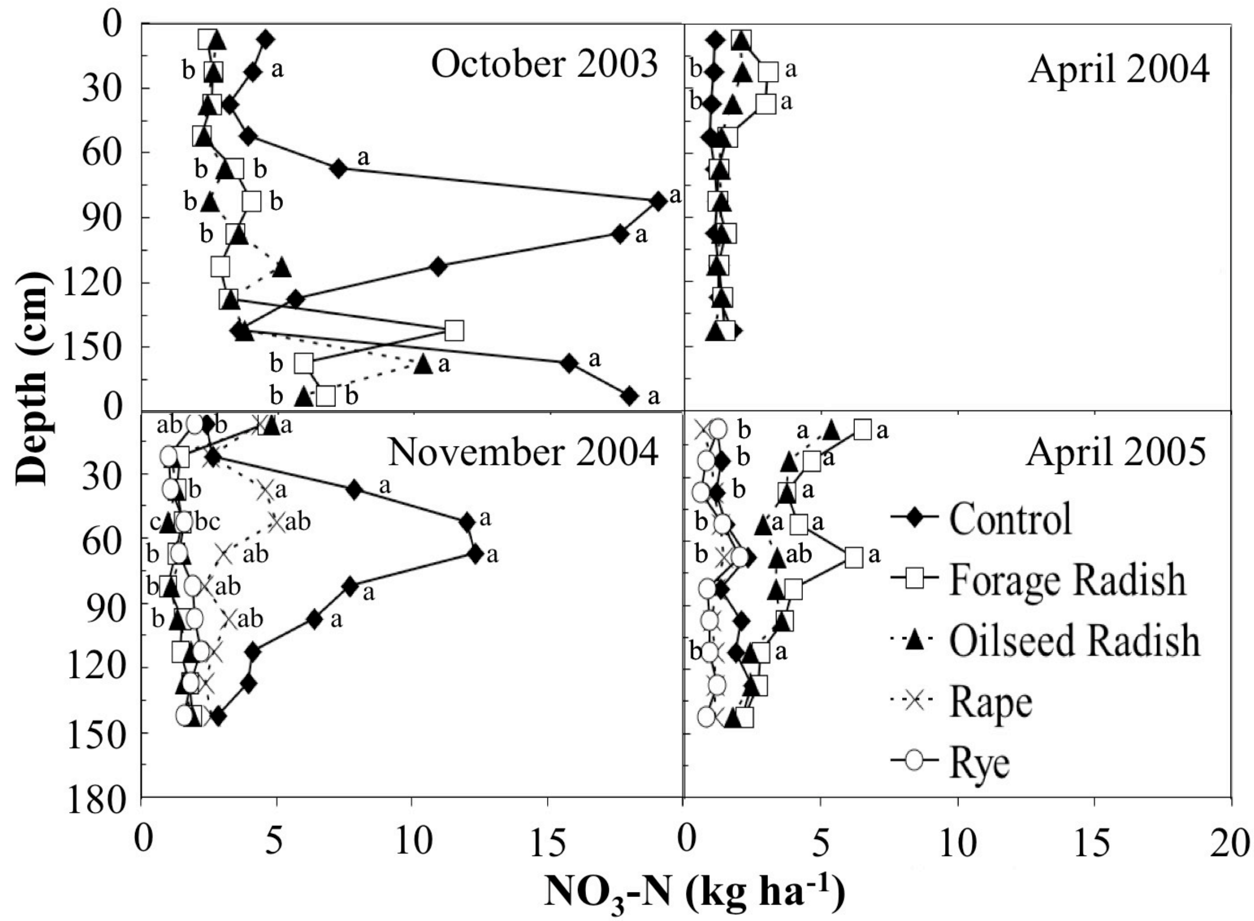

Figure 1.3. Soil nitrate-nitrogen for each 15 cm depth increment sampled at University of Maryland Wye Research and Education Center (WREC) from November 2003 through May 2005. Small letters indicate significantly different means within each depth, p<0.1.

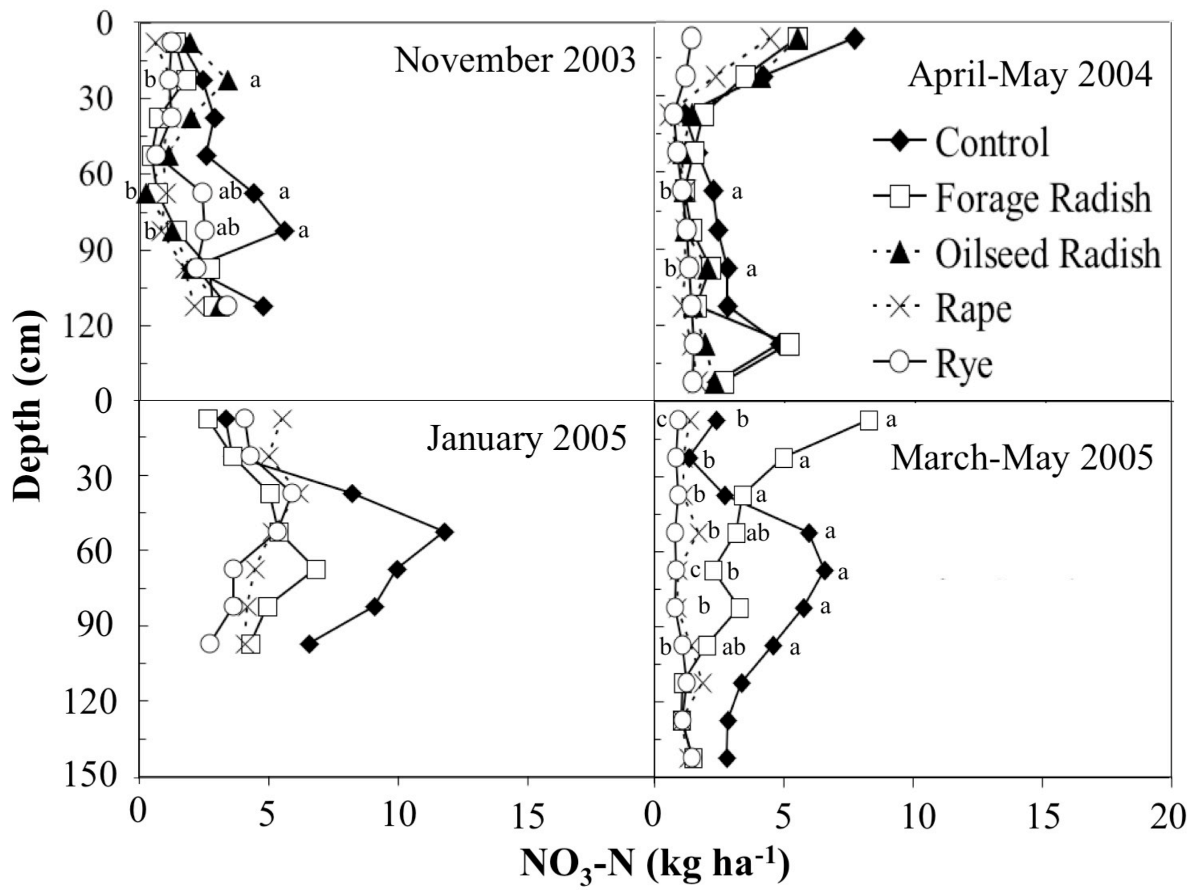

Figure 1.4. Soil porewater nitrate-nitrogen at University of Maryland Central Maryland Reearch and Education Center-Beltsville facility (CMREC) and Wye Research and Education Center (WREC) in the spring of 2004 and 2005. One lysimeter was installed for each treatment plot in spring 2004, and two lysimeters were installed in each plot in spring 2005. Lysimeters were sampled weekly. Small letters indicate significantly different means for each date sampled, p<0.1.

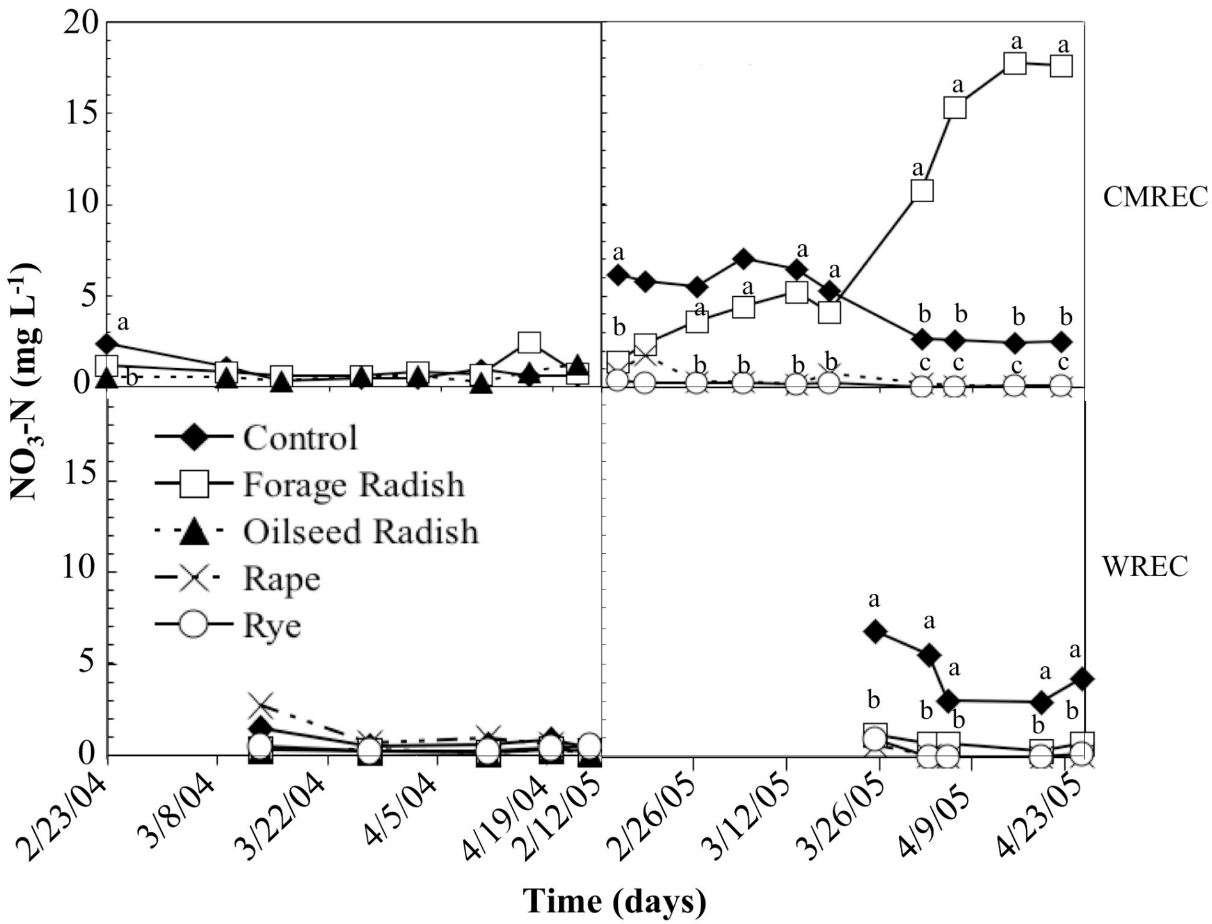

Table 1.4. Estimated mineral N changes over the 150 cm soil profile at University of Maryland Central Maryland Research and Education Center-Beltsville facility (CMREC1) from October 2003-April 2004. Negative values indicate a loss from the system. Small letters indicate significantly different means, $p<0.1$.

	Total Plant Fall N Uptake	Soil Profile Mineral N (0-150 cm)		Mineral N Changes From Oct 03-April 04†	Apparent Mineral N Changes + Total Plant N Uptake
		Fall	Spring		
Treatment	kg N ha^{-1}	kg N ha^{-1}		kg N ha^{-1}	kg N ha^{-1}
Control		196a	48.6a	-147a	-147a
Forage radish	127a	124a	38.6a	-157a	-30b
Oilseed radish	143a	141a	52.6a	-143a	0b

† equation: (spring soil NO_3-N for treatment plot) - (fall soil NO_3-N for control plot)

Table 1.5. Mineral N (ammonium-nitrogen plus nitrate-nitrogen) and nitrate-nitrogen changes in soil profile (0-150 cm) compared to weedy fallow control plots at University of Maryland Central Maryland Research and Education Center-Beltsville facility (CMREC2) from November 2004-April 2005. Negative values indicate a loss. Small letters indicate significantly different means within column, $p<0.1$.

	Total Plant Fall N Uptake	Total Plant Spring N Uptake	Soil Profile NO_3-N (0-150 cm)		NO_3-N Changes from Nov 04-April 05†	Soil Profile Mineral N (0-150 cm)		Mineral N Gains From Nov 04-April 05‡
			Fall	Spring		Fall	Spring	
Treatment	kg N ha^{-1}	kg N ha^{-1}	kg NO_3-N ha^{-1}		kg NO_3-N ha^{-1}	kg N ha^{-1}		kg N ha^{-1}
Control			65.0a	21.0bc	-44.0b	95.5a	126a	30.5a
Forage radish	214a		19.5c	43.9a	-21.1a	40.6bc	171a	75.5a
Oilseed radish	214a		19.2c	38.5ab	-26.5a	42.5bc	188a	92.5a
Rape	152b	106	35.1b	12.3c	-52.7b	62.1b	128a	32.5a
Rye§	65.6	73.0	18.5c	14.3c	-50.7b	31.2c	125a	29.5a

† equation: (fall soil NO_3^{-}N for control plot) – (spring soil NO_3^{-}N for each treatment plot)
‡ equation: (spring soil mineral N for each treatment plot) – (fall soil mineral N for control plot)
§ data on shoots only

Table 1.6. Mineral N (ammonium-nitrogen plus nitrate-nitrogen) gains and changes in soil profile (0-105 cm) compared to weedy fallow control at University of Maryland Wye Research and Education Center (WREC2) from January 2005-May 2005. Negative values indicate a loss. Small letters indicate significantly different means within column, p<0.1.

	Total Plant Fall N Uptake	Total Plant Spring N Uptake	Soil Profile NO_3-N (0-105 cm)		NO_3^-N Changes from Nov 04-April 05†	Soil Profile Mineral N (0-105 cm)		Mineral N Changes From Nov 04-April 05‡
			Fall	Spring		Fall	Spring	
Treatment	kg N ha^{-1}	kg N ha^{-1}	kg NO_3-N ha^{-1}		kg NO_3-N ha^{-1}	kg N ha^{-1}		kg N ha^{-1}
Control			56.4a	31.7a	-24.7a	148a	160a	12a
Forage radish	215a		22.0b	28.1a	-28.3a	151a	162a	11a
Rape	192a	138	18.2b	9.93b	-46.5b	150a	160a	10a
Rye§	99.0	83.0	16.8b	6.75b	-49.7b	142a	140a	-2a

† equation: (fall soil NO_3^-N for control plot) – (spring soil NO_3^-N for each treatment plot)
‡ equation: (spring soil mineral N for each treatment plot) – (fall soil mineral N for control plot)
§ data on shoots only

CHAPTER IV

BRASSICA COVER CROP INFLUENCE ON DISSOLVED AND SOLUBLE ORGANIC NITROGEN IN MARYLAND COASTAL PLAIN SOILS

ABSTRACT

Evidence that terrestrial dissolved organic N losses as well as mineral N losses contribute to surface water degradation has caused reassessment of nutrient cycling in natural and agricultural systems. This study examined the influence of the Brassica cover crops forage radish (*Raphanus sativus* L cv. 'Daikon'), oilseed radish (*Raphanus sativus* L. cv. 'Adagio'), rape (*Brassica napus* L. cv. 'Dwarf Esssex'), and the small grain rye (*Secale cereale* L. cv. 'Wheeler') on soil and porewater organic N in two agricultural sites in the mid-Atlantic coastal plain: Beltsville and Wye. Soil porewater was collected weekly in lysimeters in spring of 2004 and 2005 for dissolved organic N (DON) monitoring. Soil samples (0-15 and 90-105 cm depths) were collected once in fall and once in spring from 2003-2005 and extracted with 0.5 M K_2SO_4 to quantify surface and subsoil soluble organic N (SON). Neither DON nor SON was affected by cover crops. Fluctuations in porewater DON coincided with precipitation and plant decomposition. Seasonal variability was observed in surface SON while subsoil SON contents remained relatively constant, and SON amounts decreased with increasing depth. Cover crop treatment effects were observed in the DON proportion of total dissolved N (TDN) and SON proportion of total soluble N (TSN) at Beltsville due to changes in inorganic N. Cover crops had little effect on organic N losses, and altered the organic proportion of the TSN and TDN pools by affecting soil mineral N. The two soils of contrasting texture had substantially different organic N losses.

INTRODUCTION

Researchers have traditionally assumed that most leaching losses of N were in mineral forms (mainly as nitrate) and therefore historically, nitrate-N has been the focus of most nutrient studies (Shipley et al., 1992; Sainju et al., 1998; Isse et al., 1999). Nitrate-N is known to have negative effects on human and animal health, as well as contribute to eutrophication of surface waters. Recent research, however, has begun to elucidate the roles of dissolved organic N in nutrient cycling, plant nutrition, and environmental impacts. Literature suggests that dissolved organic N in natural ecosystems may be utilized directly by plants (Okamoto and Okada, 2004; Jones and Kielland, 2004; Jones et al., 2005a; Jones et al., 2005b), but not necessarily in amounts that significantly contribute to either plant nutrition or decreases of organic N in the soil or the soil solution (Jones et al., 2005a). A net loss of organic N from natural ecosystems as dissolved organic N (DON) (Qualls et al., 1991; Qualls et al., 2000; Perakis and Hedin, 2002) affects surface water quality by the uptake of DON by aquatic algae with or without mineralization (Brookshire et al., 2005). Phytoplankton in surface waters are capable of absorbing DON, which may result in harmful algal blooms (Glibert et al., 2004; Glibert et al., 2001; Mulholland et al., 2004).

The organic N pool may be divided into two broad classifications: DON and soluble organic N (SON) (Murphy et al., 2000). Organic N found in soil porewater is termed DON whereas the organic N that is associated with soil particles and extractable by water or salt solution is SON (Murphy et al., 2000). Dissolved organic N is broad-ranging in form, from low molecular weight (LMW) amino acids and amino sugars to recalcitrant phenolic compounds (Jones et al., 2004; Smith, 1987). Amino acids and amino sugars are a minor proportion of DON however since plants and microorganisms easily take up these LMW compounds and remove them from soil solution, possibly contributing directly to mineralization rates of N (Jones et al., 2004). After plant and microbial consumption of the LMW portion of DON, studies suggest the biodegradability of DON may be low as marine DON was observed to be resistant to microbial degradation (Peierls and Paerl, 1997; Stepanauskas et al., 1999). Soluble organic N is the pool of easily mineralisable (Mengel et al., 1999), hydrophobic, organic N usually adsorbed to mineral horizons (Tipping et al., 1999; Kaiser et al., 1997). Soluble organic N is composed of the more labile forms of N in the soil such as amino acids, amino sugars, and humic N compounds (Dick et al., 2000) that are easily utilized by plants and microorganisms (Jones et al. 2004). In agricultural soils the SON reservoirs may be comparable in size to mineral N pools (Jensen et al., 1997; Bhogal et al., 2000) with DON only 10% of the SON on average (Murphy et al., 2000). The size of the organic N pools becomes greater with less land disturbance (Christou et al., 2005; Zhu and Carreiro, 2004; Brodowski et al., 2004). Christou et al. (2005) measured DON in the 0-15 cm soil layer of 94 agricultural sites to be 57±8% of the total dissolved N (TDN) in soil solution. Ninety-four percent of the leaching N in a deciduous forest soil was found to be organic N (Qualls et al, 1991).

Research on mineral N in agroecosystems indicates system losses of this N pool may be decreased by manipulating field management, crop rotations, and timing and rates of fertilizer application (Meisinger et al., 1991; Weinert et al., 2002; Burgess et al., 2002). Current literature does not suggest that the amount of organic N in soil or leaching water may be as easily manipulated to limit losses, especially in the short term.

Appel and Mengel (1990, 1992) found no effect on the amount of SON by rape (*Brassica napus* L.) in sandy soils. Amino acid-specific cotransporters in maize (*Zea mays* L.) roots may absorb SON (Jones and Darrah, 1994), but still preferentially take up mineral N even in low N conditions (Okamoto and Okada, 2004). Sorghum (*Sorghum bicolor* Moench) and rice (*Oryza sativa* L.) grown in low mineral N soils are capable of using organic N as a key nutritional pathway (Okamoto and Okada, 2004). *Arabidopsis*, a member of the family Brassicaceae, has also been noted to have the ability to capture organic N depending on soil pH and amino acid concentrations within the plant (Fischer et al., 2002). Intense competition with soil microorganisms and the availability of mineral N species limit uptake of organic N by agronomic crops (Jones et al., 2005a).

This study assessed the influence of several Brassica species and rye on SON and DON pools in two agricultural soils with both mineral and organic N inputs. Quantity and seasonal variability of SON in both surface and subsoil samples, and DON in porewater were measured, and the proportions of SON and DON in the total nitrogen pools in soil and porewater were determined.

MATERIALS AND METHODS

Field experiments were conducted at University of Maryland Research and Education Center at Beltsville facility (CMREC) and Wye Research and Education Center (WREC) from August 2003-May 2005. The experimental design was a randomized complete block with 4 replications.

CMREC Site Description

The well drained soils at the CMREC site were a complex of Cedartown and Evesboro loamy sands formed from coastal plain sediments averaging 17 mg organic matter g^{-1} soil and pH 6.5 in the surface horizon (0-10 cm). The soil in two of the experimental blocks is dominated by Evesboro loamy sand (mesic, coated Lamellic Quartzipsamment) with 78% sand and 6% clay in the surface horizon, and clay content of less than 6% from the bottom of the Ap horizon to a depth of 120 cm. The dominant soil in the other two blocks is Cedartown loamy sand (siliceous, mesic Psammentic Hapludult) with a surface horizon texture of 82% sand and 7% clay. Clay content in the argillilc horizon ranges from 15-20% clay (80 cm to 120 cm). A seasonal perched water table during the winter and early spring is suggested by redoximorphic features below 90 cm depth in the Cedartown loamy sand. Mean annual precipitation at CMREC (39.02° N, 76.53° W) is 1112 mm and mean annual temperature is 12.8°C. Precipitation events and average daily temperatures at CMREC during the study are shown in Figure 2.1.

CMREC Site Management

The soil was last plowed on March 31, 1999, and a crop rotation of corn (*Zea mays* L.) / winter wheat (*Triticum aestivum* L.) / double crop soybean (*Glycine max* (L.) Merr.) with zero-tillage management was introduced at that time until August 2003. For the duration of this study, a corn / full-season soybean rotation was maintained. Treatment plot dimensions were 3.7 m by 9.1 m.

This experiment used plots and treatments within a larger experiment that had plots for both drilled and broadcast cover crop plots. Plots used during fall 2003-spring

2004 (CMREC1) for cover crop treatments were not used for the second year of the experiment in fall 2004-spring 2005 (CMREC2). The plots used during the second year were embedded within the larger experiment, but had a failed stand (zero biomass) of broadcast cover crops during fall 2003-spring 2004. Both CMREC1 and CMREC2 had no prior cover crop rotation although both sets of plots were subjected to the same main crops during the study.

<u>Year 1 Study (CMREC1)</u>

On August 13, 2003, forage radish (*Raphanus sativus* L cv. Daikon) and oilseed radish (*Raphanus sativus* L. cv. Adagio) (13 kg ha^{-1} seeding rate) were no-till drilled (15 cm row spacing) into wheat stubble and fertilized with 56 kg ha^{-1} of N as $(NH_4)_2SO_4$ for cover crop nutrition at CMREC1. Forage and oilseed radishes winterkilled during December 2003-January 2004. Glyphosate (1.1 L ha^{-1} AI; N-(phosphonomethyl)-glycine) was applied to the entire field on May 11, 2004 for weed control. Glyphosate resistant soybeans (cultivar NK S39-Q4) were planted on May 12, 2004 (155,000 seeds ha^{-1}; 38 cm row spacing) and glyphosate (1.1 L ha^{-1} AI) was again applied for weed control on June 24, 2004. Soybeans were mowed on August 18, 2004 in the CMREC2 plots (reproductive stage R8) to provide an organic source of N (~ 200 kg N ha-1) that would allow evaluation of cover crop N uptake potential the following fall and winter.

<u>Year 2 study (CMREC2)</u>

Forage and oilseed radishes, rape (*Brassica napus* L. cv. Dwarf Essex), and rye (*Secale cereale* L. cv. Wheeler) (seeding rates, 13, 13, 10, and 126 kg ha^{-1}) were no-till drilled on August 25, 2004, into the mowed soybean plots. Forage and oilseed radishes winterkilled in December 2004-January 2005. Rape and rye continued to grow through the spring and were terminated on April 27, 2005 using glyphosate (2.3 L ha^{-1} AI). Lime was applied at a rate of 1120 kg ha^{-1} on May 5, 2005, followed by corn planting (Pioneer 34B62; 65,000 seeds ha^{-1}; 76 cm row spacing) on May 10, 2005, without starter fertilizer. The corn was side dressed with N fertilizer in mid-June after the last soil samples were collected.

WREC Site Description

The soil at WREC is a somewhat poorly drained Mattapex silt loam (fine-silty, mixed, active, mesic Aquic Hapludult) formed from loess deposits overlaying coastal plain sediments. The surface horizons averaged 27% sand, 18% clay, 20 mg organic matter g^{-1} soil and pH 6.3. An argillic horizon (26% clay) was observed from 30-50 cm depth with conversion to the coarser coastal plain sediments (loam-loamy sand) occurring between 100-120 cm depth. Mean annual precipitation at WREC (39.03° N, 76.04° W) was 1103 mm and mean annual temperature was 13.3°C. The daily precipitation and daily average temperature for WREC is shown in Figure 2.1.

WREC Site Management

The soil at WREC was in no-till management for this experiment and the previous 5 years with a corn/full-season soybean rotation. Plots used from fall 2003 through spring 2004 (WREC1) had forage radish, oilseed radish, rape and rye cover crops planted since August 2001. Plots used in fall 2004 through spring 2005 (WREC2) had not supported cover crops since 1983 (no information available before 1983). The two sets of plots used at WREC were adjacent to one another in location and had no differences in soil profile characteristics. Treatment plot dimensions were 3.2 m by 9.1 m with 15 cm row spacing.

Year 1 (WREC1)

Glyphosate (0.93 L ha^{-1} AI) was applied on August 1, 2003, at WREC1 for weed control in preparation for the planting of forage radish, oilseed radish, rape, and rye (seeding rates, 14, 14, 10, and 126 kg ha^{-1}) on August 19, 2003. Forage and oilseed radish winterkilled during December 2003-January 2004 while rape and rye were terminated by rolling and application of glyphosate (1.4 L ha^{-1} AI) at WREC1 on May 5, 2004. Glyphosate-resistant soybeans (cultivar NK S39-Q4) were no-till drilled (155,000 seeds ha^{-1}; 76 cm row spacing) at WREC1 and WREC2 on May 21, 2004. Glyphosate (1.9 L ha^{-1} AI) was sprayed for weed control within the soybeans on June 23, 2004. Soybeans in the WREC2 plots were mowed on September 18, 2004, for cover crop fertilization (~250 kg N ha^{-1}) to examine the following cover crop N uptake potential.

Year 2 study (WREC2)

Forage radish, rape, and rye were planted (14, 10, 126 kg ha^{-1}, seeding rates) on September 24, 2004, into the mowed soybean plots. Forage radish winterkilled in December 2004-January 2005, and rape and rye were terminated using glyphosate (1.9 L ha^{-1} AI) on May 3, 2005. Corn (Pioneer 34B62) was no-till drilled (40,000 seeds ha^{-1}) on May 19, 2005, without starter fertilizer. The corn was side dressed with N fertilizer in mid-June after the last soil samples were collected.

Field sampling

Soil samples were collected from late October through November, and March through May of each year. Soil samples (up to 180 cm depth) were obtained using a Veihmeyer corer and drop hammer (Devera et al., 1980; Veihmeyer, 1929). Soil cores were placed in 4 cm diameter PVC troughs for examination and dividing into depth increments. Three cores were taken per plot, divided into 15 cm increments, and homogenized to represent the plot. Bulk density for each increment was calculated using the known diameter of the Veihmeyer corer's cutting tip. Because of possible physical disturbance at the soil surface, average bulk density for each site and season was calculated for the 0-15 cm (average 7.5 cm) depth. The average bulk density for each site over the entire experiment was calculated for the 90-105 cm (average 97.5 cm) depth. The 0-15 cm increments and 90–105 cm were analyzed for NO_3-N, NH_4-N, and total soluble N (TSN). Soil NO_3-N and NH_4-N concentrations were subtracted from TSN concentration to determine SON for both sampled depths. The bulk density values were used to calculate N as g cm^{-3} soil.

Tension lysimeters (1.9 cm inner diameter) were installed one per plot in February 2004 at CMREC1 and WREC1, and two per plot in February 2005 at CMREC2 and WREC2. Lysimeters at CMREC1 were installed to a depth of 90 cm in spring 2004, and to a depth of 120 cm at CMREC2 in spring 2005. At WREC1, lysimeters were installed to a depth of 75 cm in March 2004. Lysimeters were install to a depth of 90 cm at WREC2 in March 2005. Soil porewater was collected weekly from lysimeters using a hand-held pump (80 to 90-kPa vacuum applied for 24-48 hours) from installation through the end of April in 2004 and 2005. Samples were immediately acidified with 4 M H_2SO_4 for preservation followed by storage at -20°C. Analysis of porewater NO_3-N and TDN was conducted and DON concentration calculated by subtracting NO_3-N from the TDN concentration. Porewater was analyzed for NH_4-N on selected samples but was not detectable. Monthly leaching water volumes were calculated using the Thornthwaite method (Dunne and Leopold, 1978) (Equation 1), which estimates evapotranspiration (E_t) from average monthly temperature.

(1) $$E_t = 1.6\left[\frac{10Ta}{I}\right]^a$$

where E_t = potential evapotranspiration (cm mo^{-1})

Ta = mean monthly air temperature (° C)

I = annual heat index = $\sum_{i=1}^{12}\left[\frac{T_{ai}}{5}\right]^{1.5}$

a = 0.49 + 0.01791 – 0.0000771I^2 + 0.000000675I^3

A correction factor for latitude (40°N) was multiplied by the E_t in March (0.99) and April (1.10) to adjust for the number of days per month and the length of the day. Precipitation data was used to calculate the percolating water volume assuming no surface runoff. Evapotranspiration was subtracted from the percolating water volume to determine the volume of soil porewater transporting NO_3-N through the soil (leaching volume). Leaching volume was multiplied by the monthly average concentration of NO_3-N in soil porewater to obtain an approximate weight per area leaching loss amount (kg ha^{-1}).

Laboratory analyses

Soil samples were extracted using 0.5 M K_2SO_4 (1:10 dilution). Analysis of NH_4-N in soil extracts and porewater was conducted using an ammonia gas selective electrode (Orion EA940; Thermo Electron, Waltham, MA) (Banwart et al, 1972) after addition of ionic strength adjusting solution, which raised the pH of each sample to 13. A copperized cadmium reduction column and flow-injection autoanalyzer (Technicon Autoanalyzer II; Technicon Industrial Systems, Tarrytown, NY) were used for soil and porewater NO_3-N analyses (Technicon method no. 487-77a). Soil extracts and porewater samples were digested using alkaline persulfate. Soil extracts were autoclaved (Cabrera and Beare, 1993) and porewater samples were microwaved (He et al. 1990) following addition of alkaline persulfate, and analyzed colorimetrically for TSN and TDN (Cataldo et al., 1975). The digestion method was changed from microwaving the samples to autoclaving because greater numbers of samples could be processed at a time. Both methods yielded recovery greater than 90% for amino acids and greater than 80% for nicotinamide adenine dinucleotide (NAD^+). Soluble organic N and dissolved organic N were calculated as:

(2) $$\text{SON} = \text{TSN} - (NO_3\text{-N} + NH_4\text{-N} \text{ in extract})$$

(3) $$\text{DON} = \text{TDN} - (NO_3\text{-N} \text{ in water})$$

Negligible amounts of NH_4-N (<0.1 mg L^{-1}) were initially measured in all water samples, so this parameter was subsequently measured only for selected sample dates to confirm that levels were neglible in porewater samples. .

Statistical analysis

Statistical analyses for soil and porewater samples were conducted using SAS version 9.1 (SAS Institute, Cary, NC). Repeated measures and split-plot in time ANOVA for porewater samples were conducted using Proc Mixed to compare the average DON concentrations over the entire sampling period. Analysis of variance was used to compare DON concentration for treatments on each sampling date. Soil samples were compared using ANOVA at each depth for each season and split-split plot ANOVA over the length of the experiment with treatment as main plot, site as a subplot, and time as a sub-subplot. Block effects were treated as a random variable while treatments were considered fixed effects. Tukey Multiple Mean Comparison tests and orthogonal contrasts were used to compare treatment differences and differences between depth within a treatment. Due to the limitations on the number of subsamples obtained per plot (3 cores per plot), a p-value < 0.10 was considered significant.

RESULTS

Porewater DON

Brassica and rye cover crops had no significant effect on soil porewater DON concentrations overall or on any individual sample date (Figure 2.2). Fluctuations in porewater DON concentrations coincided with precipitation events and were more dynamic within the coarser soils at CMREC compared to WREC. CMREC1 had similar DON leaching losses in March and April of 2004 (7.2 and 6.1 kg ha^{-1} mo^{-1}), but dramatically increased from March to April 2005 (5.3 and 31.6 kg ha^{-1} mo^{-1}) at

CMREC2. WREC1 and WREC2 had similar leaching losses during April of 2004 and 2005 (3.9 and 4.3 kg ha^{-1} mo^{-1}).

The fraction of DON in the porewater samples fluctuated over the spring sampling season. Dissolved organic N was 72% of TDN from February 24 through March 27, 2004 (6 cm rainfall from February 6-March 27), and decreased to 42% of TDN from April 3-23, 2004 (9 cm rainfall from April 1-23), at CMREC1. Treatment by time interaction occurred at CMREC2 in spring 2005 (Figure 2.3) with rape and rye plots having significantly higher fractions of DON within porewater than control and forage radish plots over the sampling period (average of 57% and 23-26%). No treatment effects were evident at WREC1 and WREC2. Changes in the percentage of DON over time were significant at WREC1 between April 11 and 19, 2004, (Figure 2.4) corresponding with precipitation events (6.3 cm rain from April 11-14). The fraction of DON in TDN at WREC2 averaged 27% over the sampling season.

Soil SON

Cover crops did not significantly affect soil SON in surface or subsoil samples, but the surface soil had significantly higher SON compared to the subsoil (Figure 2.5). At CMREC1 and CMREC2, SON for each depth was relatively constant throughout the experiment, with an average of 24.3 kg ha^{-1} at the 0-15 cm depth and 13.3 kg ha^{-1} at the 90-105 cm depth. In surface soil at WREC1 and WREC2, SON differed significantly between fall and spring of each sampling year. Subsurface SON amounts from fall 2004 through spring 2005 were quite similar. In fall 2003, there was no significant difference in SON amount between the surface and subsoil (36.8 kg ha^{-1}) at WREC1.

The portion of soil SON in TSN was influenced by treatment and depth as well as time. A significant difference in the SON fraction between the surface and subsoil was evident at WREC2 from fall 2004 through spring 2005 (Figure 2.6). Surface SON proportions did not significantly fluctuate over the experiment at WREC. Subsoil SON fraction was lower in the experimental plots used from fall 2004 through spring 2005 (WREC2) compared to those used from fall 2003 through spring 2004 (WREC1) (Fig. 2.6). A treatment effect on the SON portion occurred in fall at CMREC1 and CMREC2 (Fig. 2.7). All cover crop plots had a significantly higher percentage of SON compared to control in fall while rape and rye plots had higher proportions of SON in spring 2005 compared to the radish plots which winterkilled (contrasts, $p<0.06$).

DISCUSSION

Mineralization of forage radish tissues had already begun by the time of sampling in the spring (Figure 2.8, 2.9) and no significant increases in either soil SON or porewater DON were detected. Precipitation and soil textural influences appear to be major controlling factors in the leaching of DON, with coarser soils having larger fluctuations over time and greater concentrations within porewater compared to finer textured soils. Jensen et al. (1997) observed seasonal fluctuations in surface SON on coarse textured soils, which did not occur at CMREC, but did occur on the finer-textured soil at WREC. Surface horizons (0-10 cm) were found to have the greatest amounts of organic N as also observed by Woods (1989).

Decomposition of the radishes is quick and well underway in March through May thereby releasing N into the TSN and TDN (Figure 2.8) pools. The subsequent mineralization of organic N decreases the SON and DON proportions especially in coarser soils. DON proportions were 10-20% greater during March for the radishes than April when mineral N losses increased. Mineral N leaching losses were greatest in spring 2005 (>55% mineral N) following soybeans and corresponding with reductions in SON at all depths in radish plots. Low precipitation at CMREC1 decreased leaching and nitrogen limitations allowed for little release of mineral N into the soil or porewater as a result of microbial immobilization in spring 2004. Leaching losses at CMREC1 contained 70% DON which was a stark contrast to CMREC2 leaching losses (28-38% DON in control and radishes). Radishes at WREC had higher DON proportions in leachate over both study years compared to CMREC. The overwintering rape and rye had > 80% DON in leachate in April 2005 at CMREC2 and WREC2, which is consistent with observations by Chapman et al. (2001) that vegetation increases DON in leachate.

The range of SON amounts in the surface soil reported in this experiment is in agreement with the body of published research. Surface SON at CMREC ranges from 15-30 kg ha^{-1} over the experiment, similar to the values reported by Jensen et al. (1997) for a loamy sand. In fall 2003, CMREC was coming out of a corn-soybean-winter wheat rotation and surface SON (18 kg ha^{-1}; 54-63% of TSN) corresponds with the SON amount reported by McNeill et al. (1998) for soil under wheat and is also within the range of the SON portion of TSN. The finer textured Mattapex soil at WREC ranged from 30-63 kg ha^{-1} in the 0-15 cm layer with SON composition from 56-73% of TSN, slightly larger amounts than those reported by Mengel et al (1999) and Murphy et al (2000). Subsoil SON showed less seasonal variability than surface SON as Bhogal et al. (2000) also found. Excluding WREC1 in fall 2003, subsoil SON at WREC was comparable to that measured by Bhogal et al. (2000) in cultivated and reseeded grasslands on a silty clay loam. SON amounts decreased with increasing depth as similarly reported by Bhogal et al. (2000), and Patra et al. (1999) under grassland. In WREC1 and WREC2, the decrease in SON corresponded with an increase in mineral N with increasing depth, which may be explained by partial mineralization of SON in the subsoil as proposed by Patra et al. (1999) and the movement of NO_3-N from surface to deeper soil layers. Subsoil SON proportions of the TSN in the coarser-textured soil at CMREC were similar to surface SON which supports the findings of Patra et al. (1999) that lower rates of mineralization of SON are associated with sandy soils due to low organic matter sorption**,** and the increased mobility of SON to deep soil layers in coarser soils by Bhogal et al (2000).

Surface SON decreases at WREC2 contrasted sharply with trends observed at other sites in this study. Surface mineral N at WREC2 was much greater than at other sites in fall, enhancing the rate of mineralization as also observed by Azam et al. (1993), and mineralization of soybean tissues was found to be greater in fine-textured soils by Pare and Gregorich (1999), which may explain why surface SON decreases were not observed at CMREC2 following soybeans.

Even in fall 2004 when a large amount of organic N was added to plots from mown soybeans, the finer textured soil at WREC2 had similar SON and DON losses to the previous year (WREC1) when no fertilizer was applied. Larger DON losses were observed at CMREC2 after fall 2004 with similar amounts of SON in the soil compared to the previous year. With the 200 kg N ha^{-1} addition to CMREC2 from the mown soybeans and little change in the soil SON, this may indicate that some N may have been lost by DON leaching quickly through the well-drained, coarse soil at CMREC2, denitrification, or a combination of the two processes. Coarser soils were found to have less ability to conserve organic N by cation exchange processes as compared to finer textured soils (Dashman and Stotzky, 1982; Kaiser and Zech, 2000) which supports the conjecture that DON may have been lost through leaching processes.

Treatment effects on percentage of SON in the TSN amount result from the uptake of mineral N or by release of SON during tissue decay, increasing the amount of soil SON relative to mineral N in those plots. The continued growth and uptake of mineral N by rape and rye in the spring increased the SON and porewater DON proportions in those plots compared to the radishes that winterkill (Figure 2.8). There were no significant correlations among soil SON, porewater DON, and porewater NO_3-N in present data, even on dates when both soil and porewater samples were collected. Murphy et al. (1998) also found little relationship among these parameters.

Seasonal fluctuations of subsoil SON at WREC1 were opposite those trends at WREC2, CMREC1 and CMREC2 (Figure 2.5). Subsoil SON was much greater than surface SON in fall at WREC1, and a large decrease in SON from fall to spring followed. Subsoil SON at WREC2, CMREC1, and CMREC2 over the study year were relatively constant and lower than surface SON unlike at WREC1. Forage radish plots contained 119 kg SON ha^{-1} in the subsoil and largely influenced the average subsoil SON at WREC1 in fall (average without forage radish plots was 22.5 kg ha^{-1}; average with forage radish plots was 45.8 kg ha^{-1}). Subsoil SON proportions of the TSN pool at WREC1, CMREC1 and CMREC2 were over twofold greater than that of WREC2 in spring even though spring subsoil SON amounts were similar among sites. Brassica and rye cover crops have been shown to reduce soil mineral N (Meisinger et al., 1991; Kristensen and Thorup-Kristensen, 2004) without having any effect on SON therefore several years of cover cropping may have increased the ratio of SON to mineral N in subsoil through efficient recycling at WREC as was found by Staver and Brinsfield (1998) with multiple years of rye cover cropping.

While surface and subsoil SON increased from fall to spring, mineral N decreased, especially in the deep layers of the control plots (loss of 14.9 kg ha^{-1}). As NO_3-N concentrations in porewater increased or decreased there was relatively little inverse relationship with DON dynamics in cover crop plots (Figure 2.8, 2.9), probably due to the diversity of tissues, the diversity of organisms participating in decomposition and mineralization, and the varying rates that they perform these processes. Nitrate-N and DON in porewater collected from control plots, however, had a solid inverse relationship (Figure 2.8, 2.9) perhaps due to lack of additional contributions from plant tissue during fall, winter, and spring. Porewater data indicate that DON is as dynamic as porewater NO_3-N in its fluctuations over spring.

The SON and mineral N pools in soil are not static because N is continuously transformed. The N transformation cannot be fully addressed using just two sampling times (once in spring and once in fall) as in this experiment.. Porewater N pools were monitored more frequently during the spring and the transformations between DON and mineral N become more clear (Figure 2.8, 2.9). Porewater from control plots shows most clearly the transformations occurring as the labile fractions of DON are mineralized to NO_3-N. Porewater collected from cover crop plots indicates there is a more complex relationship between the organic and mineral N pools where crops are growing or where recent plant tissue has been added from winterkilled crops. Release of N from a variety of plant tissues in different stages of decomposition mask the N transformations occurring in the cover crop plots, at least in the broad sense of organic N. Examining the various classes of organic N in DON or SON would likely elucidate the N transformations taking place in soil and soil solution.

CONCLUSIONS

Cover crops are major N recyclers in agroecoystems. Although little evidence indicates that agricultural crops take up organic N directly, crops are the main suppliers of organic N by capture of mineral N, conversion to amino forms, and release of organic N into the soil through tissue decomposition and root exudates. Since agronomic crops preferentially capture mineral forms of N, there was a larger direct affect on the organic N proportions rather than total mass in soil and porewater. There was no discernible relationship between plant uptake and SON increases from fall to spring.

Although cover crop effects on SON and DON amounts were not observed in this experiment, several trends were obvious. Subsoil SON appears not to fluctuate as greatly as does surface SON, perhaps due to the sorption of SON to soil aggregates may increase SON resistance to mass flow and affect susceptibility to mineralization (Vinolas et al., 2001; Vieublé Gonod et al., 2006) compared to DON. Crop effects on DON and SON proportions were not consistent from year to year or between sites. Dissolved organic N and SON were greater proportions of TDN and TSN at CMREC (59% of TDN, 67% of TSN averaged over 2004 and 2005 sampling periods) than WREC (44% of TDN; 55% of TSN). The DON pool as a percentage of SON was greater following soybeans, and was greater at CMREC than at WREC over both study years (DON at CMREC1 and CMREC2 was 15-47% and 54-87% of SON; DON at WREC1 and WREC2 was 8-27% and 8-57% of SON) (Figure 2.2, 2.5). The proportion of DON in leachate was typically greater from rape and rye in spring (>70% DON) due to continued growth and mineral N uptake than control and radishes (15-68% DON). Leaching losses of DON were lower at WREC than at CMREC in spring, and related to differences in soil organic matter, texture, and drainage. Relationships between SON and DON fluxes and proportions were not observed in the data collected.

Without further understanding of the nature of organic N and its dynamics, a means of manipulating organic N losses (if there are means) from agricultural lands cannot be developed. Given evidence that DON inputs to marine and freshwater bodies of water is contributing to eutrophication, the importance of understanding the relationships between SON, DON, soil organic matter, and mineral N increases. Past research on decreasing nutrient loads to the Chesapeake Bay have focused solely on nitrate-N, but our data suggests that organic N constitutes a substantial proportion of the N lost by leaching. Estimates of N loading and changes in N leaching due to management practice may therefore have been subject to considerable error by not considering dissolved organic N.

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Figure 2.1. Daily precipitation and daily average temperature at University of Maryland Central Maryland Research and Education Center-Beltsville facility (CMREC) and Wye Research and Education Center (WREC) from August 2003 through May 31, 2005.

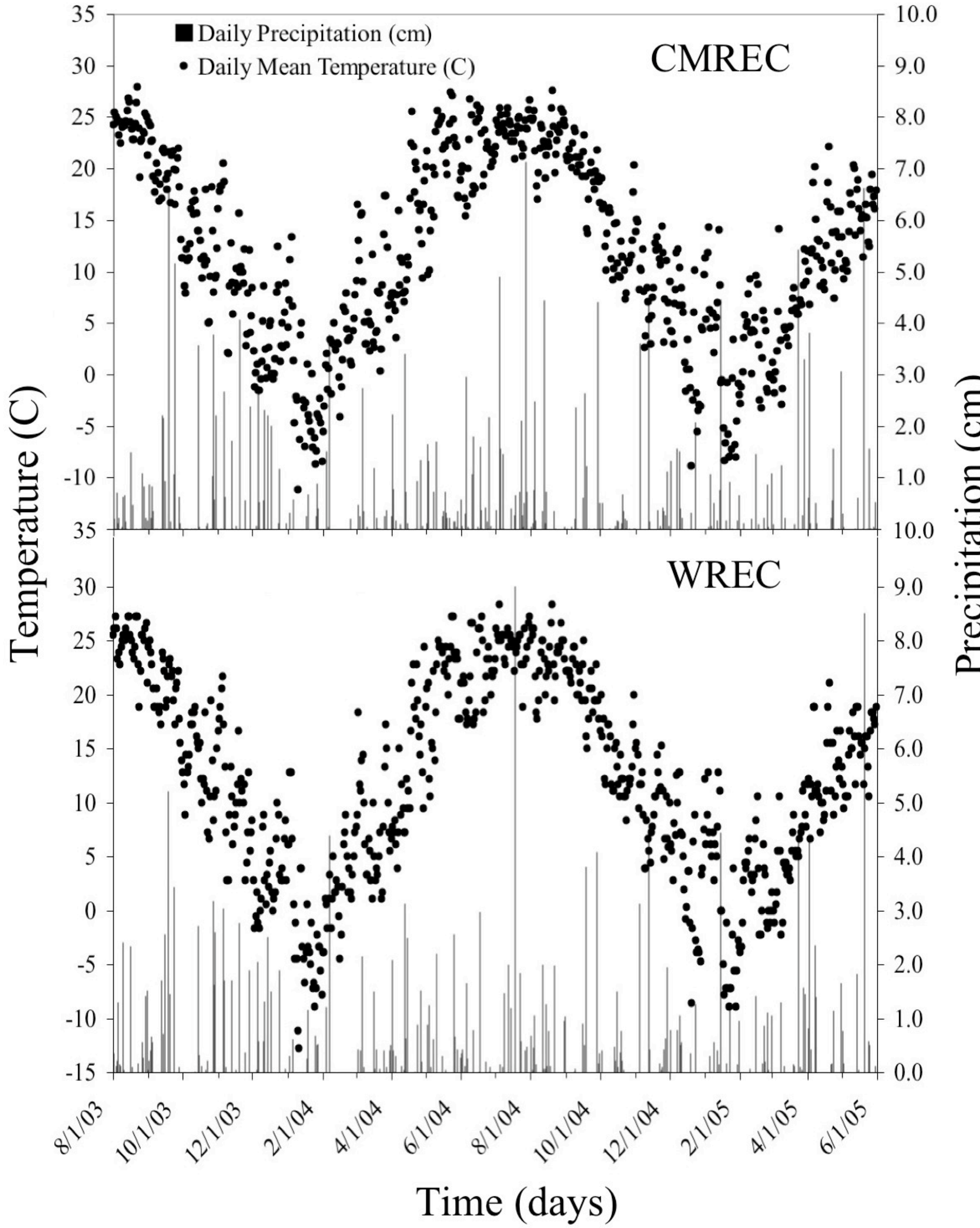


Figure 2.2. Soil porewater dissolved organic N (DON) at University of Maryland Central Maryland Research and Education Center-Beltsville facility (CMREC) and Wye Research and Education Center (WREC) in spring of 2004 and 2005. One lysimeter was installed per treatment plots in spring 2004 (75 cm depth at WREC; 90 cm depth at CMREC), and two lysimeters were installed per plot in spring 2005 (90 cm depth at WREC; 120 cm depth at CMREC). Porewater samples were collected weekly from lysimeters.

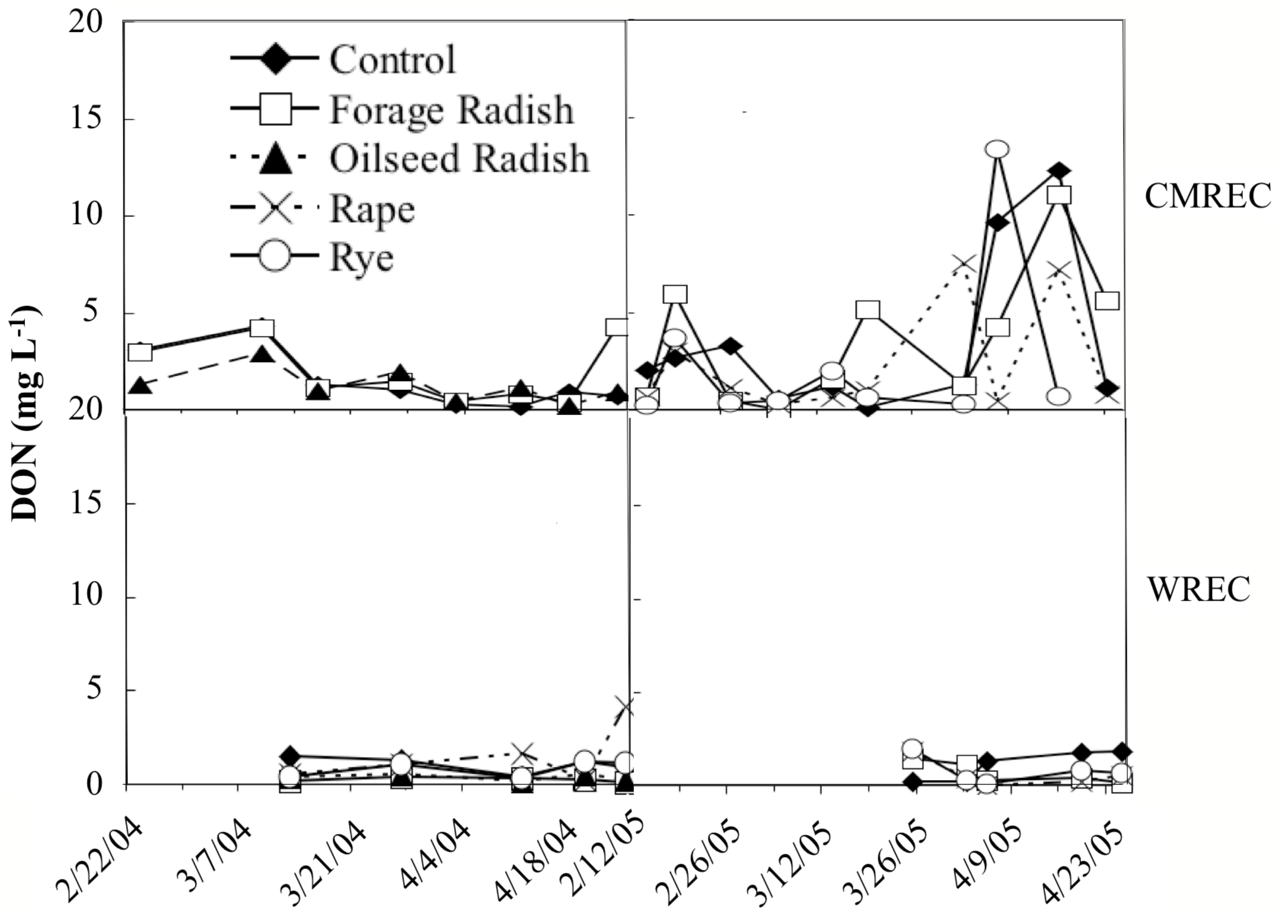


Figure 2.3. Fraction of dissolved organic N (DON) in the total dissolved N (TDN) concentration of soil porewater at University of Maryland Central Maryland Research and Education Center-Beltsville facility (CMREC2) in spring 2005. Porewater was collected weekly from lysimeters installed at 120 cm depth in treatment plots (two per plot). Small letters indicate pairwise significant differences between treatment within sampling date, p<0.1.

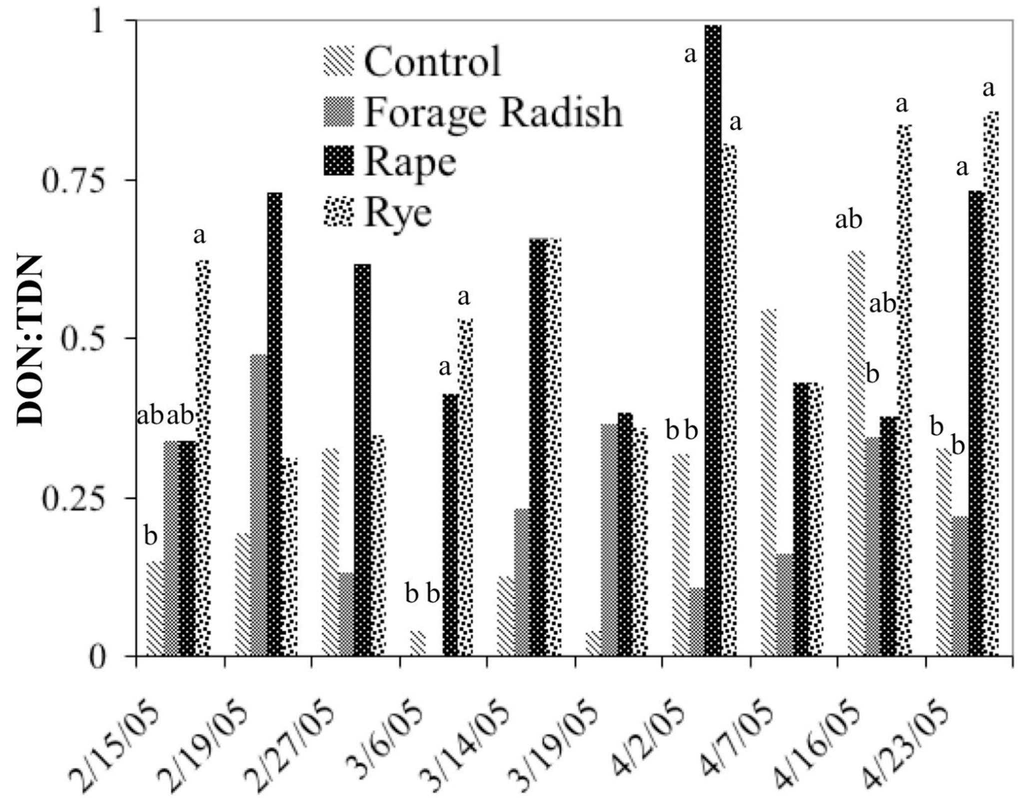


Figure 2.4. Fraction of dissolved organic N (DON) in the total dissolved N (TDN) concentration in soil porewater at University of Maryland Wye Research and Education Center (WREC) in spring 2004. Means across all cover crop treatments. Porewater was collected weekly from lysimeters (two per plot) installed to a depth of 90 cm.   Small letters indicate pairwise significant differences, p<0.1.

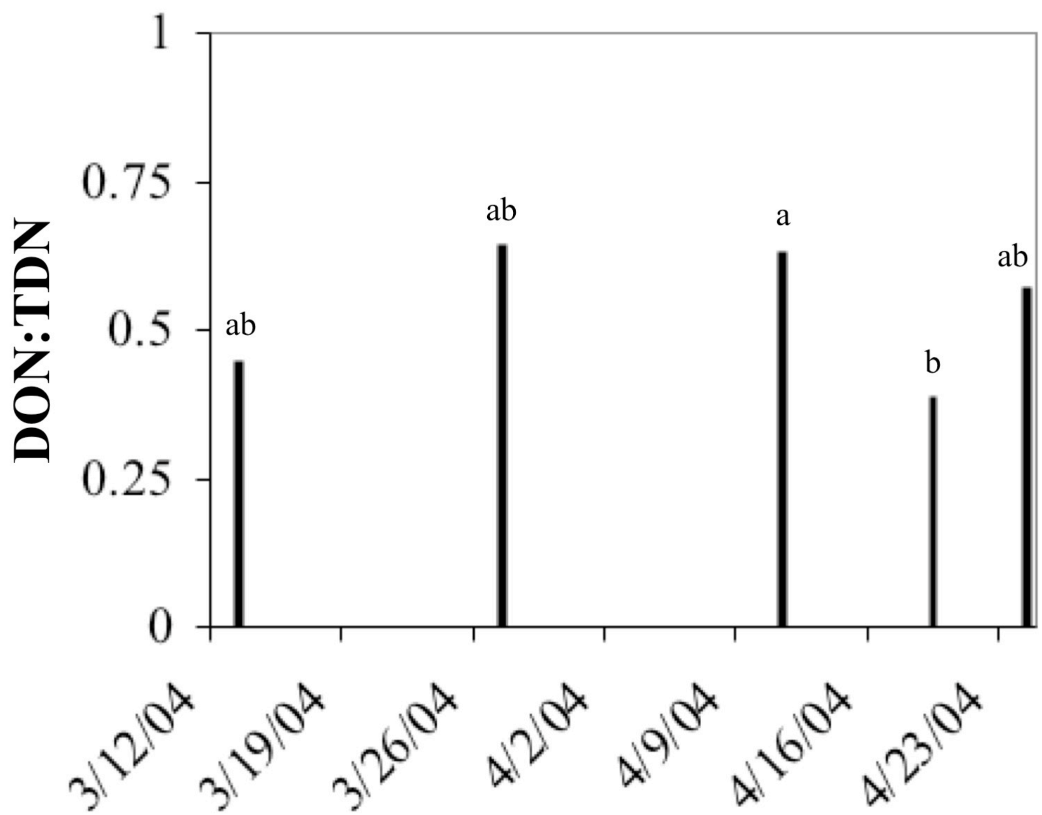


Figure 2.5. Soil soluble organic N at University of Maryland Central Maryland Research and Education Center-Beltsville facility (CMREC) and Wye Research and Education Center (WREC) at average depths of 0-15 cm and 90-105 cm. Results at WREC1 in Fall 2003 represent only 2 blocks. Fall soil sampling occurred from October through January of each year. Spring soil sampling occurred from March through May of each year. Small letters indicate pairwise significant difference between depths for each season, p<0.10.

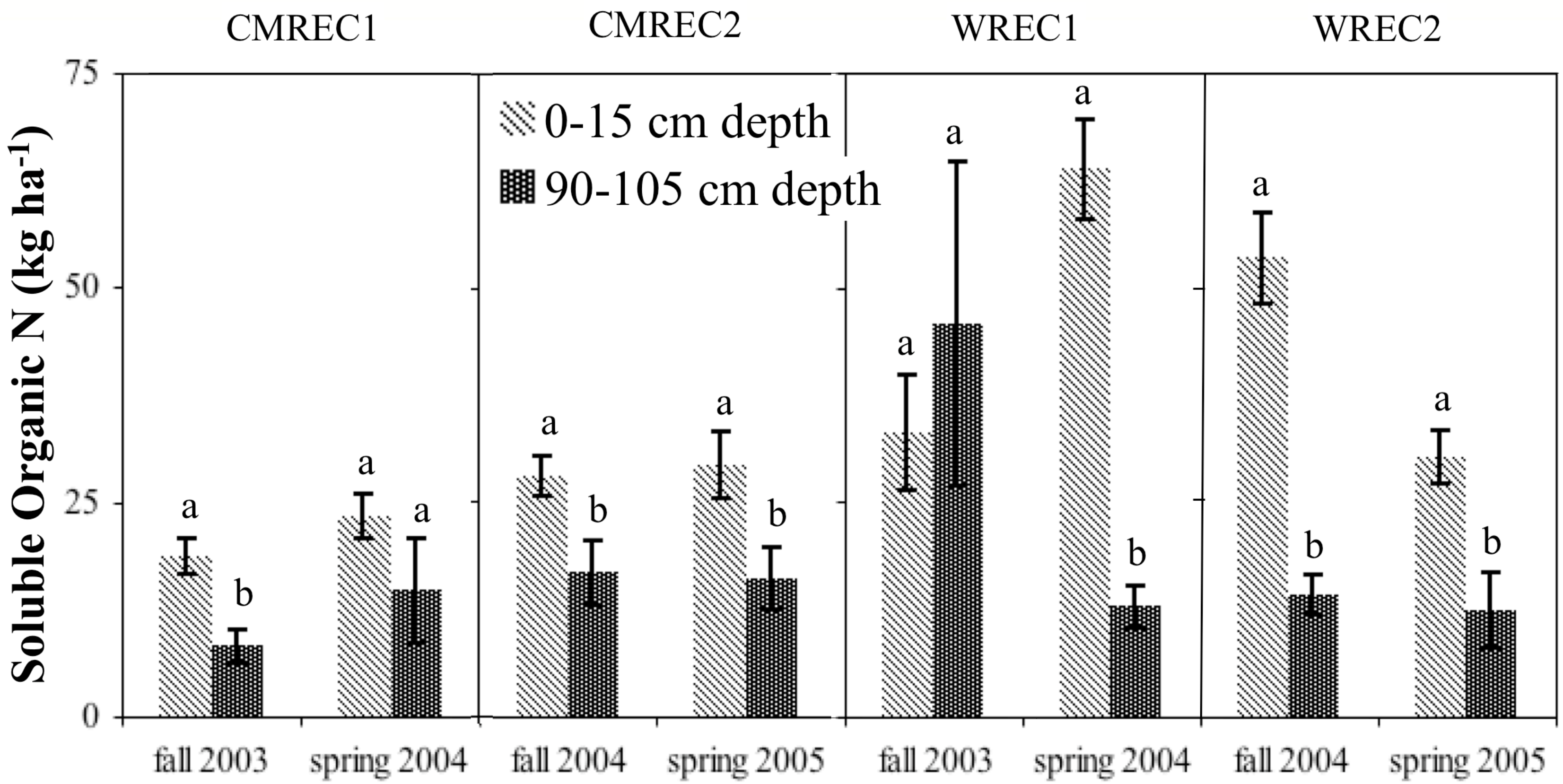


Figure 2.6. Soil soluble organic N (SON) fraction of total soluble N (TSN) at University of Maryland Wye Research and Education Center (WREC). Results at WREC1 in fall 2003 represent only 2 of 4 blocks. Soil sampling occurred from November through January and March through May. Small letters indicate pairwise significant difference between depths for each season, p<0.10

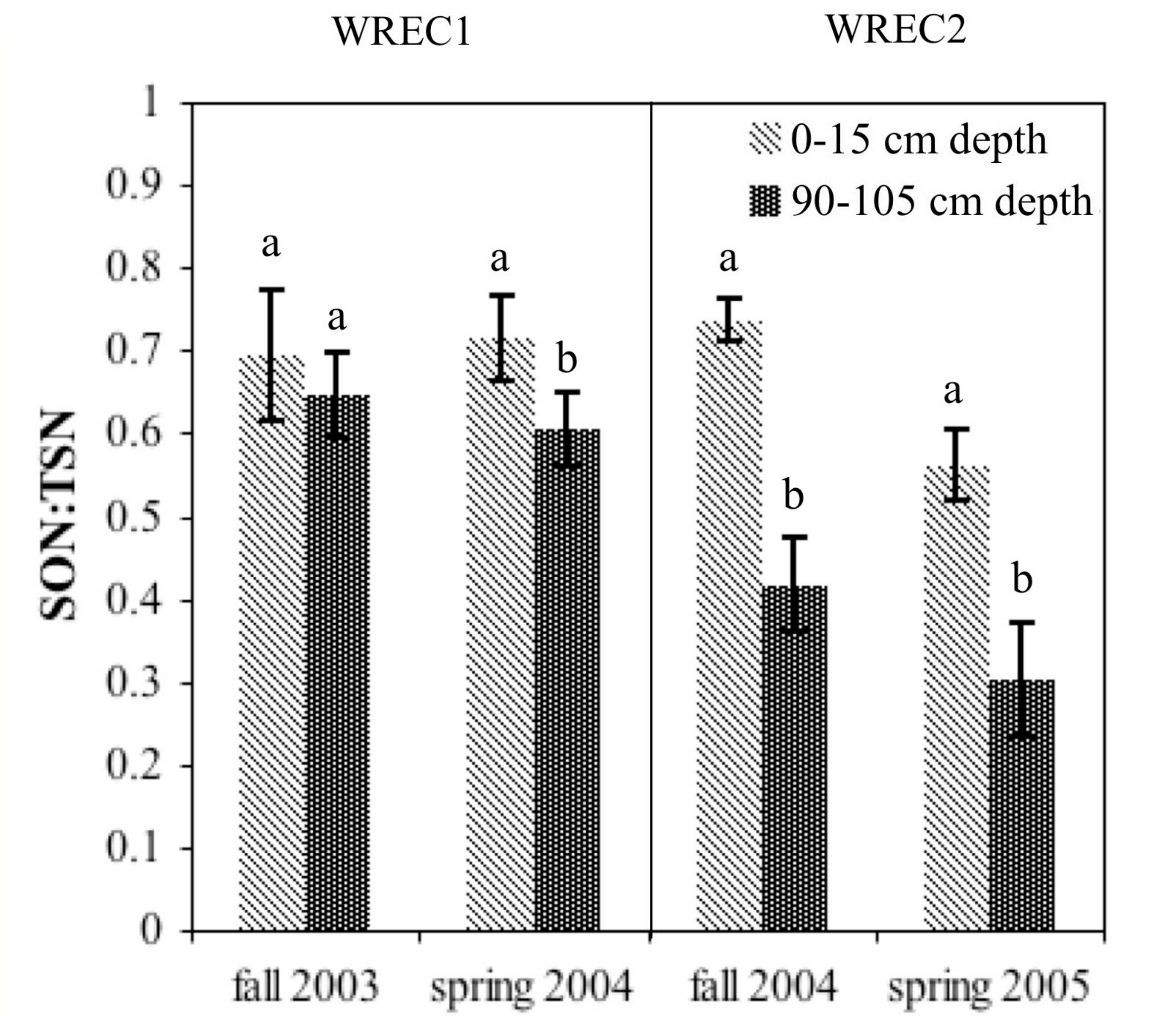


Figure 2.7. Soil soluble organic N (SON) proportion of total soluble N (TSN) at University of Maryland Central Maryland Research and Education Center-Beltsville facility (CMREC1 and CMREC2). Soil was sampled in October and March through April of each year. Results represent an average SON fraction from 0-15 and 90-105 cm depth. Small letters indicate pairwise significant difference between treatments for each season, $p<0.10$

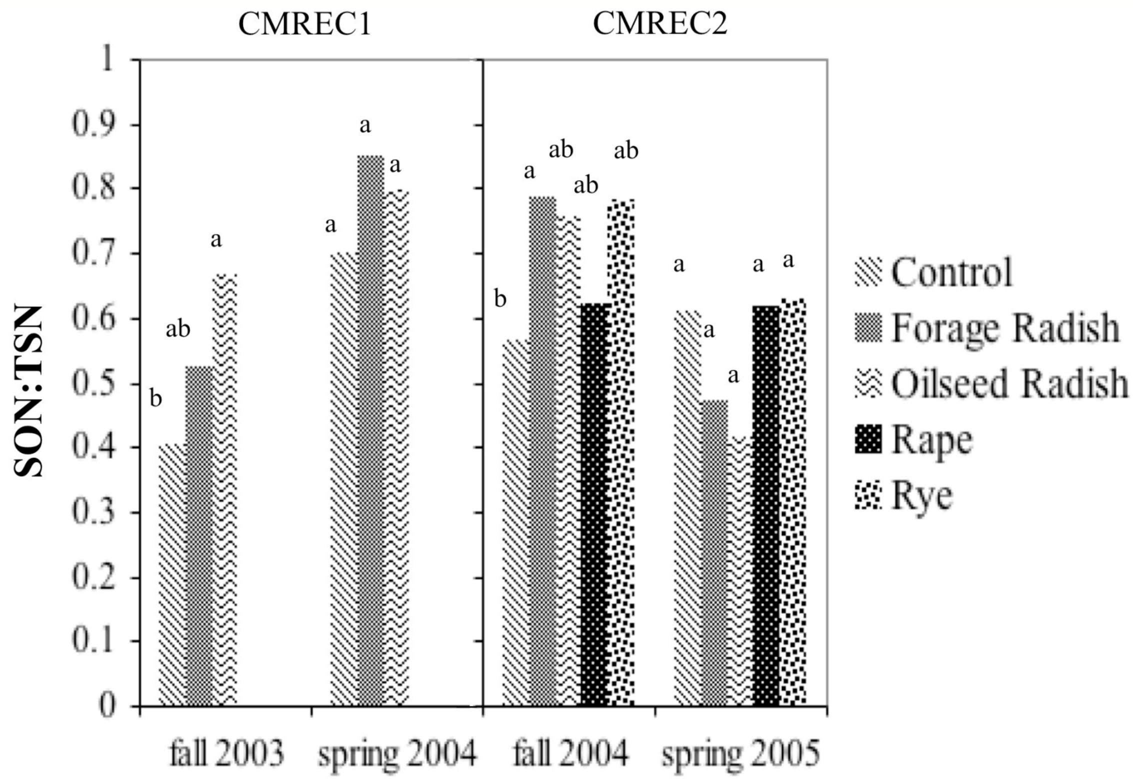


Figure 2.8. Soill porewater dissolved organic N (DON) (open symbols) and NO_3-N (filled symbols) concentrations during spring 2005 at University of Maryland Central Maryland Research and Education Center (CMREC2). Lysimeters installed to 120 cm depth (two per plot) and porewater samples were collected weekly.

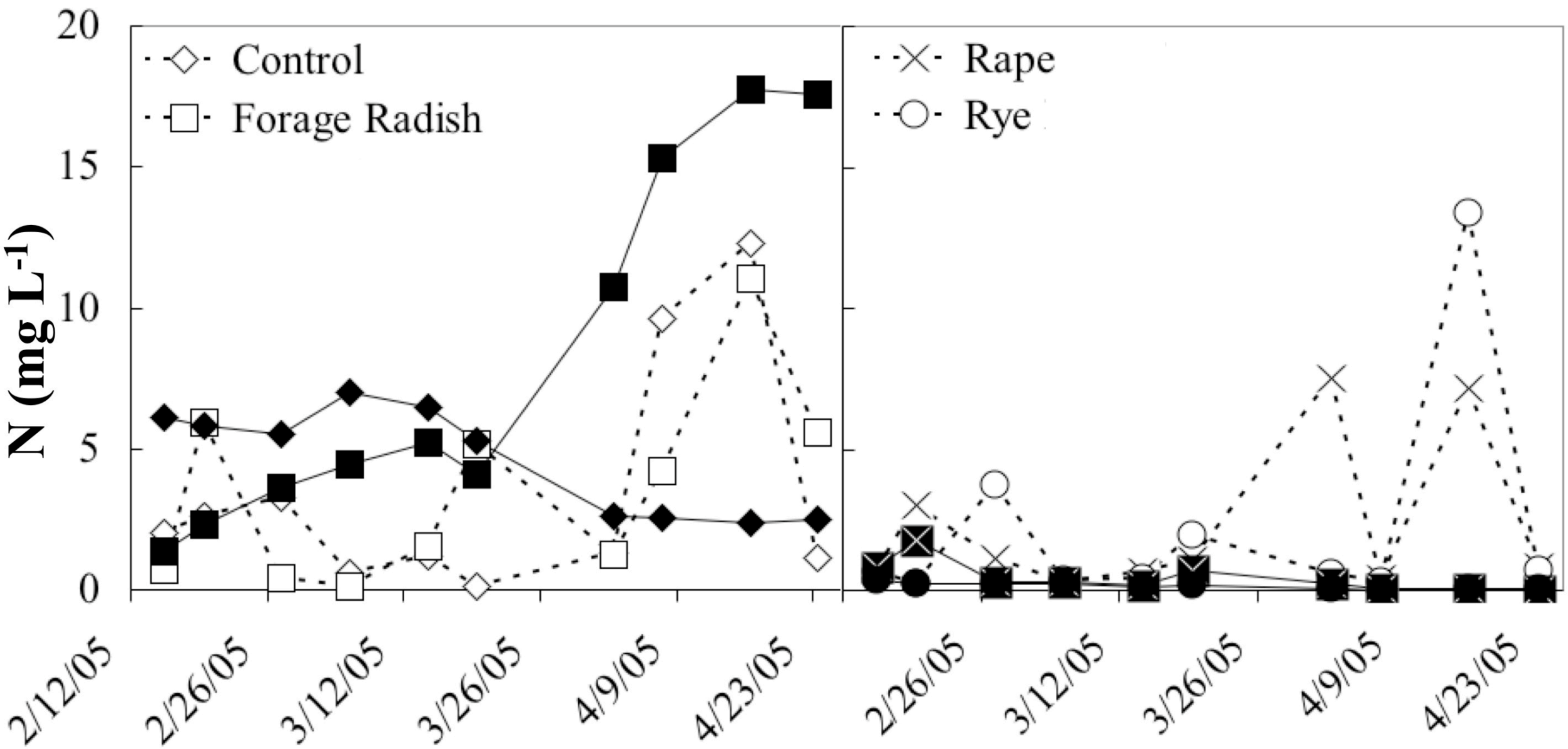
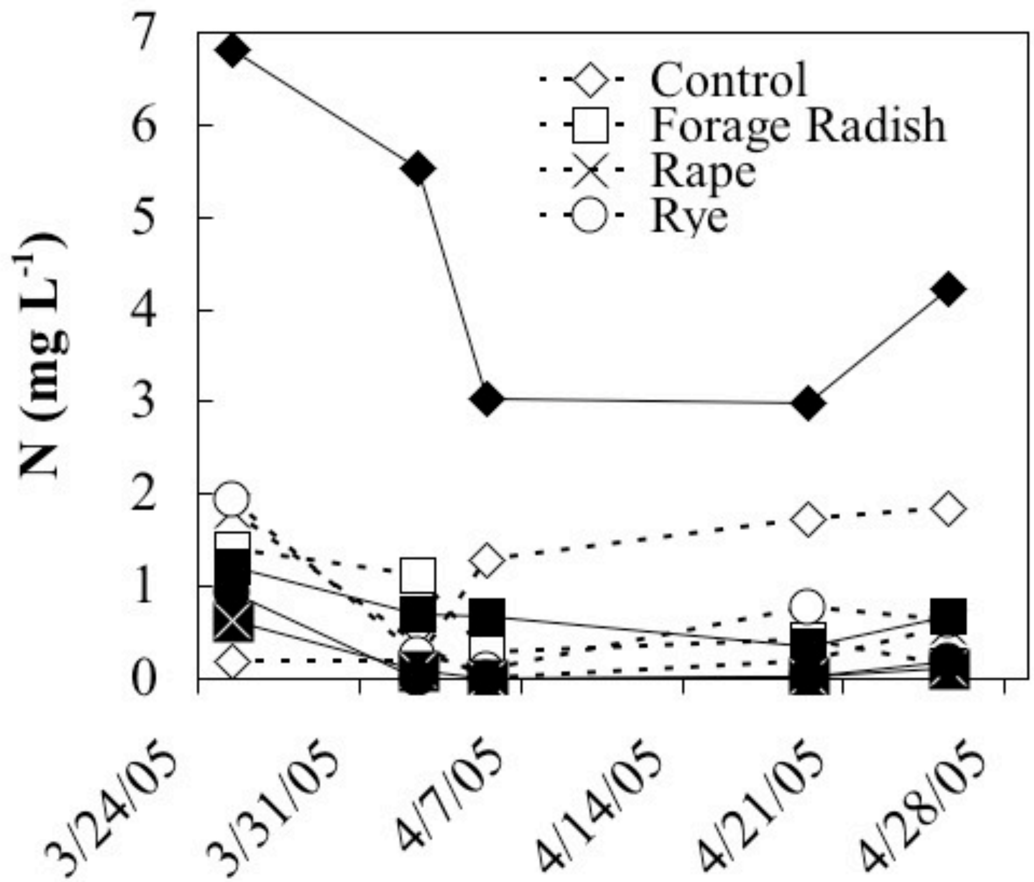


Figure 2.9. Soil porewater dissolved organic N (DON) (open symbols) and NO_3-N (filled symbols) concentrations at University of Maryland Wye Research and Education Center (WREC2) during spring 2005. Lysimeters installed to 90 cm depth (two per plot) and porewater was collected weekly.



Overall Conclusions and Recommendations

The Chesapeake Bay watershed contributed 159 million kilograms of N to the Bay in 2003 (Chesapeake Bay Foundation, 2005). Thirty-nine percent (62 million kilograms) of that N load is thought to have come from the agricultural sector. Some of the N losses from agriculture are from unused fertilizer that has leached through the soil column and into the water table, making its way slowly into the Bay. Winter and early spring are generally when N leaching is greatest since the cash crops are not growing and evaptranpiration is low. Cover crops may be planted in this interim to take up the extra subsoil N and sequester it in plant tissues until spring when the main crop is planted again. In this way, cover crops conserve N. The Maryland Cover Crop Program was instituted to help farmers with the cost of cover cropping. Farmers may be paid up to $50 for each acre of approved cover crops planted depending on the planting date. The Cover Crop Program has approved rye along with a few other small grains and rapeseed.

Rye is one of the most popular cover crops in Maryland due to its N scavenging efficiency, growth during cold weather, and adaptability to many soils (Shipley et al., 1992; Staver and Brinsfield, 1998; Coale et al., 2001). For this study, rye was used as a benchmark to compare the performance of Brassica crops for plant N uptake, soil N depletion and decreases of soil porewater NO_3^--N under crop plots in the Maryland Coastal Plain. Studies in Europe, Canada, and other regions of the US suggest that Brassicas may perform as well if not better than rye to minimize N losses (Jackson et al., 1993; Isse et al., 1999; Thorup-Kristensen, 2001; Weinert et al., 2002; Vos and Van der Putten, 2004), but with the added benefits of weed and parasitic nematode suppression, less immobilization of N, possible alleviation of subsoil compaction, and increased yields of the following main crop.

In the current study, the Brassicas forage radish, oilseed radish and rape were found to have similar if not greater plant biomass and N uptake capacity of aboveground tissues as rye in the Maryland Coastal Plain. Efficient mineral N uptake was evident by decreases in soil NO_3^--N throughout the profile sampled (105-180 cm) by Brassicas and rye. Forage and oilseed radishes winterkill and decompose throughout the early spring while rape and rye are more winterhardy and continue to grow and capture N until crop termination. On fine-textured soil, the Brassicas and rye were able to reduce soil porewater NO_3-N even in spring, but on coarse-textured soil with non-N-limiting conditions, the radishes had porewater NO_3-N concentrations greater than 10 mg L^{-1}.

Brassica root biomass and N uptake was semi-quantified in this experiment due to the ease of root sampling and cleaning for the fleshy taproot. Rye and other monocots have fine root structures which increase the difficulty of sampling and cleaning (Titlyanova et al., 1999). As in many studies, rye roots were not included in biomass or N uptake measurements (Titlyanova et al., 1999). By comparisons of soil NO_3-N depletions and aboveground tissue N uptake, it is apparent that rye roots have at least as much N uptake and storage capacity as Brassica roots.

It is unclear what influence cover cropping may have on leaching losses of organic N. No relationship between plant N uptake and decreases in DON concentrations in porewater were evident in this study. Plant N uptake, however, may increase the proportions of DON within leachate. No cover crop treatment effects on SON were found although plant uptake of mineral N increased the proportions of SON in soil. Seasonal fluctuations in SON were more prevalent in surface soil than subsoil, which remained relatively constant. Monitoring of DON indicates fluctuations throughout spring.

A challenge for future cover crop research in the Atlantic coastal plain region will be to assess the total plant N uptake including roots, for both Brassicas and rye, even though obtaining a meaningful root sample for rye is much more difficult than for the Brassicas. Cover crop N release and main crop uptake, and the role of soil texture and structure on leaching losses should also be examined. Installation of lysimeters in the fall and sampling of porewater throughout winter and spring might also prove a better indication of NO_3-N and DON leaching losses by Brassicas and rye.

With regards to organic N and agriculture, there are many areas of research that need to be addressed. How important to nutrient management is this organic N fraction? What is the full impact of organic N losses to the environment and to the bodies of water that receive the N? Can organic N losses be controlled just as inorganic N losses can be manipulated? Future research concerning SON and DON should address these questions and, hopefully, many more. Keeping in mind recent studies on organic N uptake by phytoplankton, further research on environmental impacts of organic N should be considered. By not quantifiying the entire pool of N in leaching porewater and in surface waters, a large amount of error is being incurred in measurements, calculations, and predictive models.

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APPENDICES

APPENDIX A: SOIL PROFILE DESCRIPTIONS

Soil profile descriptions for CMREC and WREC are presented. Auger borings are labeled on site layouts in Appendix B.

Site CMREC
Core # 1
Site Location outside Rep C
Soil Series Cedartown

Possible Horizon	Depth (cm)	Texture	Matrix Color	% Clay	Comment
Ap	10	ls	10YR 3.5/3	<5	
	20	ls	10YR 4/4	<5	
BA	30	ls	10YR 5/6	<5	
	40	ls	10YR 6/6	<5	
	50	ls	10YR 5/6	<5	
Bw2	60	ls	10YR 5/6	<5	15% coarse fragments (1 cm dia.)
	70	ls	7.5YR 5/6	<5	8-15% coarse fragments (2-3 cm dia.)
Bt	80	sl	10YR 6/5	10	>5% common depletions, clay skins
Bt2	90	sl	10YR 7/4	7-8	few depletions (10YR 7/3)
	100	sl	10YR 7/6	7-8	~5% common depletions (10YR 7/1), concentration color 7.5YR 6/8
BC	110	sl	10YR 6/4	5	common concentrations (7.5YR 5/8), few depletions (7.5YR 7/2)
	120	sl	10YR 6/4	5	common concentrations (7.5YR 5/8), few depletions (7.5YR 7/2)

Site	CMREC
Core #	2
Site Location	outside Rep D
Soil Series	Evesboro

Possible Horizon	**Depth (cm)**	**Texture**	**Matrix Color**	**% Clay**	**Comment**
Ap	10	ls	10YR 3/3	<5	
	20	ls	10YR 4/4	<5	
BA	30	ls	10YR 5/6	<5	
	40	ls	10YR 5/6	<5	
	50	ls	10YR 5/6	5	
Bw	60	ls	10YR 5/6	7	
	70	ls	10YR 5/6	7	
BC	80	ls	7.5YR 5/8	<5	
	90	ls	7.5 YR 5/8	<5	
BC2/2BC	100	s	7.5YR 5/8	<5	different parent material?
	110	s	10YR 7/4	<5	
	120	s	10YR 6/6	<5	

Site	CMREC
Core #	3
Site Location	outside Rep B
Soil Series	Evesboro

Possible Horizon	**Depth (cm)**	**Texture**	**Matrix Color**	**% Clay**	**Comment**
Ap	10	ls	10YR 4/4	<5	
	20	ls	10YR 4/4	<5	
BA	30	ls	10YR 5/6	<5	
	40	ls	10YR 5/6	<5	
	50	ls	10YR 5/6	<5	
Bw	60	ls	10YR 4/6	<5	
	70	ls	10YR 5/6	<5	
Bw2	80	ls	7.5YR 5/6	<5	
	90	ls	7.5YR 5/8	<5	
	100	ls	7.5YR 5/8	<5	
	110	s	7.5YR 5/6	<5	
BC	120	s	7.5YR 5/8	<5	

Site CMREC
Core # 4
Site Location between drilled and interseeded plots in Rep A
Soil Series Cedartown

Possible Horizon	Depth (cm)	Texture	Matrix Color	% Clay	Comment
Ap	10	ls	10YR 4/4	<5	
	20	ls	10YR 4/4	<5	
BA	30	ls	10YR 5/4	<5	
Bw	40	ls	10YR 6/6	<5	
	50	ls	10YR 6/6	<5	
BC	60	s	10YR 5/4	<5	
2Bw	70	ls	10YR 6/6	6	
2Bt	80	l	10YR 6/6	12	
	90	l	10YR 6/6	16	
	100	l	10YR 6/6	18	
2Bt2	110	l	10YR 7/4	20	depletions 10YR 7/1
	120	l	10YR 7/4	20	very fine sand particles, depletions 10YR 7/1

Site CMREC
Core # 5
Site Location between rep A & C in alley
Soil Series Cedartown

Possible Horizon	Depth (cm)	Texture	Matrix Color	% Clay	Comment
Ap	10	ls	10YR 3/2`	<5	
	20	ls	10YR 3/3	<5	
BA	30	ls	10YR 5/4	<5	
	40	ls	10YR 5/4	<5	
	50	ls	10YR 5/4	<5	
Bw	60	ls	10YR 6/4	<5	free water
	70	sl	10YR 6/4	12	bits of charcoal
	80	sl	10YR 6/4	15	
	90	sl	10YR 6/4	15	
Bw2	100	sl	10YR 6/6	15	distinct common depletions, 10YR 7/1
	110	sl	10YR 6/6	20	distinct common depletions 10YR 7/1, concentrations 10YR 6/8
Bg	120	sl	10YR 7/1	20	common, distinct concentrations 7.5YR 6/7, depleted matrix

Site CMREC
Core # 6
Site Location between rep B & D in alley
Soil Series Evesboro

Possible Horizon	Depth (cm)	Texture	Matrix Color	% Clay	Comment
Ap	10	ls	10YR 3/3	<5	
	20	ls	10YR 3/4	<5	
BA	30	ls	10YR 4/4	<5	
Bw	40	ls	10YR 5/6	<5	
	50	ls	10YR 5/6	<5	coarse fragments, iron store
	60	ls	10YR 5/6	<5	
	70	ls	10YR 5/6	<5	
	80	ls	7.5YR 5/6	<5	
CB	90	s	7.5YR 5/8	<5	flat coarse fragments
	100	s	7.5YR 5/8	<5	
	110	s	7.5YR 6/8	<5	
	120	s	7.5YR 7/8	<5	

Site WREC
Core # 1
Site Location between rep 1, treatment 5 & 7
Soil Series Mattapex

Possible Horizon	Depth (cm)	Texture	Matrix Color	% Clay	Comment
Ap	10	sil	10YR 4/3	18	
AB	20	sil	10YR 4/4	18	
	30	sil	10YR 4/4	18	
	40	sil	2.5Y 5/4	19	
Bt	50	sil	2.5Y 5/4	26	concentrations
	60	sil	2.5Y 5/3	26	concentrations. & depletions
	70	sil	2.5Y 5/3	26	
	80	sil	2.5Y 5/3	24	
Bg	90	sil	2.5Y 5/3	24	
	100	sil	2.5Y 7/2	24	
	110	sil	2.5Y 7/2	24	
	120	sil	2.5Y 7/1	24	

Site WREC
Core # 2
Site Location between rep 4, treatment 1 & 7
Soil Series Mattapex

Possible Horizon	Depth (cm)	Texture	Matrix Color	% Clay	Comment
Ap	10	sil	2.5Y 5/3	18	
	20	sil	2.5Y 5/4	18	
Bt	30	sil	2.5Y 6/6	23	
Bt2	40	sil	2.5Y 5/8	26	few depletions & concentrations
Bt3	50	sil	2.5Y 5/8	21	common depletions & concentrations
	60	sil	10YR 5/6	21	common depletions & concentrations
Bg	70	sil	2.5Y 7/2	17	many depletions, few concentrations
	80	sil	2.5Y 7/1	18	few concentrations
Bw	90	sil	10YR 5/1	17	no concentrations
BC	100	sl	10YR 4/6	5	few concentrations, common depletions
CB	110	ls	10YR 5/4	3	
	120	ls	10YR 5/4	3	

Site WREC
Core # 3
Site Location between drilled and interseeded plots, rep 4, treatment drilled-6 & inter-4
Soil Series Mattapex

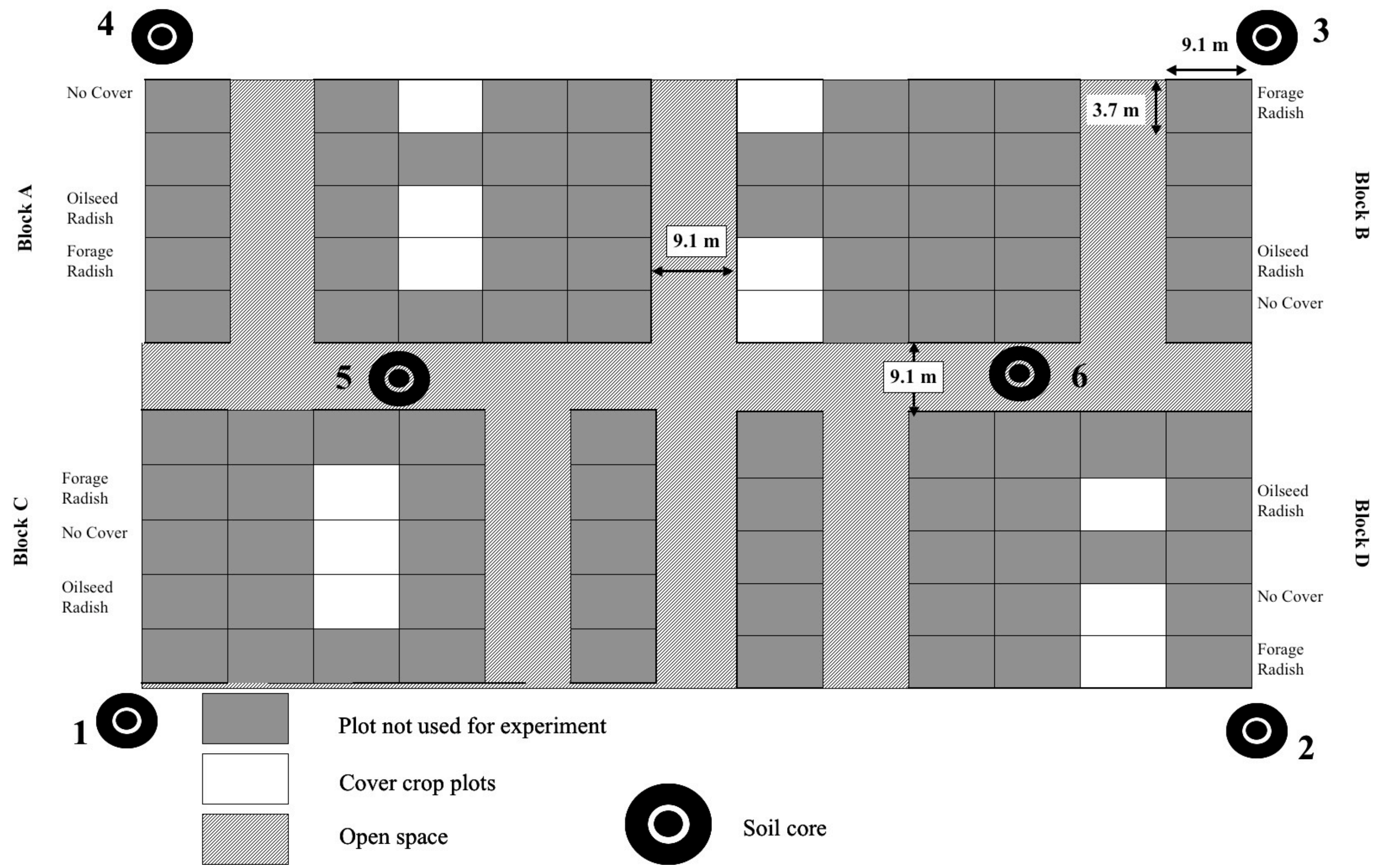
Possible Horizon	Depth (cm)	Texture	Matrix Color	% Clay	Comment
Ap	10	sil	10YR 4/4	18	
	20	sil	10YR 5/4	18	
AB	30	sil	10YR 5/4	21	
Bt	40	sil	2.5Y 5/6	25	
	50	sil	2.5Y 5/6	25	concentrations
Bw	60	sil	2.5Y 5/4	21	concentrations, some depletions
	70	sil	2.5Y 5/4	21	
	80	sil	2.5Y 5/4	21	many depletions, concentrations
Bw2	90	sil	2.5Y 6/3	21	
	100	sil	2.5Y 6/3	12	
Bg	110	sil	2.5Y 6/1	12	
	120	sil	2.5Y 6/1	12	

Site WREC
Core # 4
Site Location between drilled and interseeded plots, rep 1, treatment drilled-1 & inter-3
Soil Series Mattapex

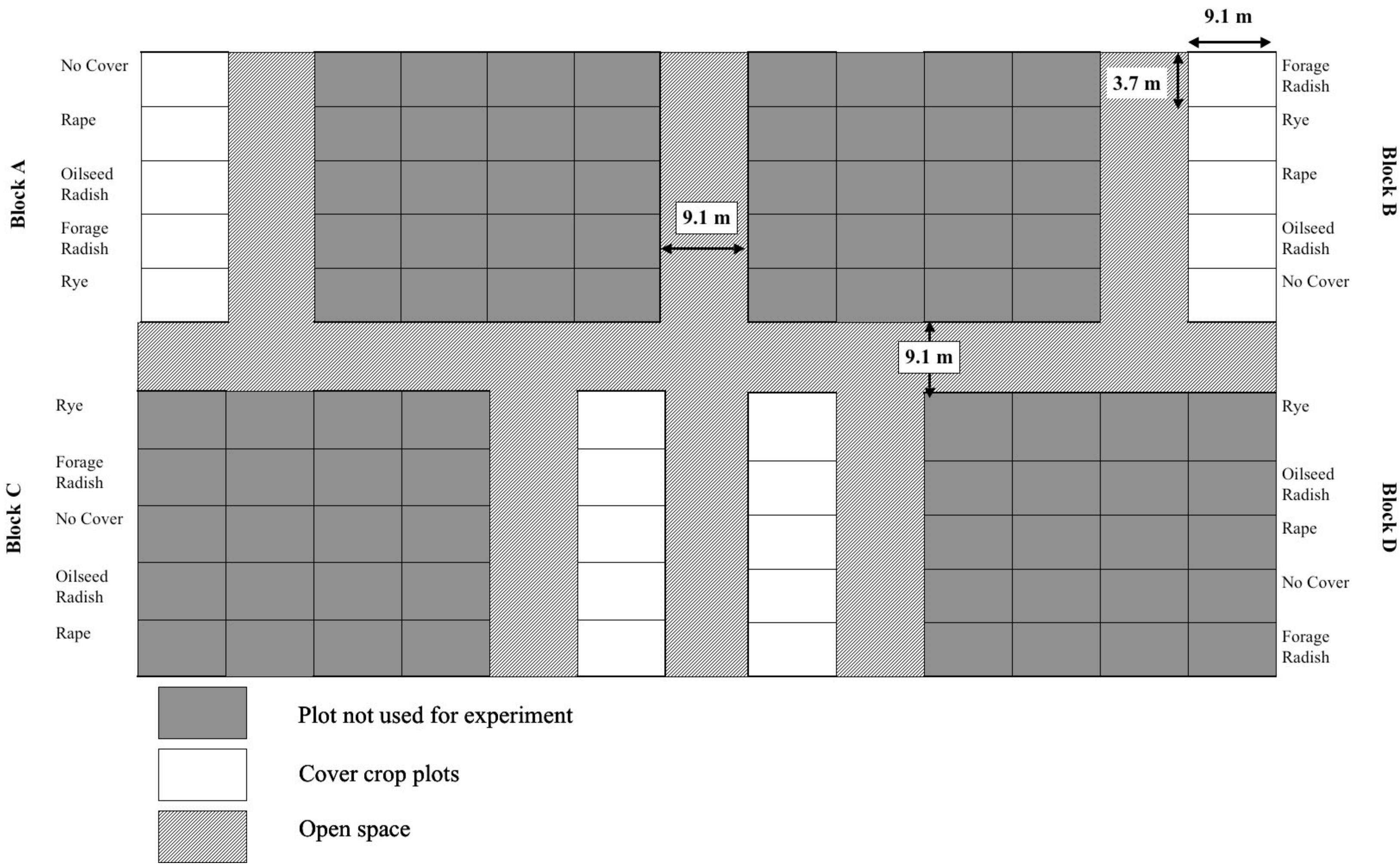
Possible Horizon	Depth (cm)	Texture	Matrix Color	% Clay	Comment
Ap	10	sil	10YR 4/4	18	
	20	sil	10YR 4/4	18	
AB	30	sil	10YR 4/4	20	
Bt	40	sil	10YR 5/6	24	
	50	sil	10YR 5/6	24	
	60	sil	10YR 5/4	26	
Bt2	70	sil	10YR 4/4	24	few depletions & concentrations
	80	sil	10YR 4/4	26	few depletions & concentrations
	90	sil	2.5Y 5/4	24	common concentrations, many depletions
Bt3	100	sil	2.5Y 5/4	30	many depletions, common concentrations
	110	sil	2.5Y 5/3	28	
	120	sil	2.5Y 4/3	28	few depletions & concentrations

Appendix B: Site Layouts

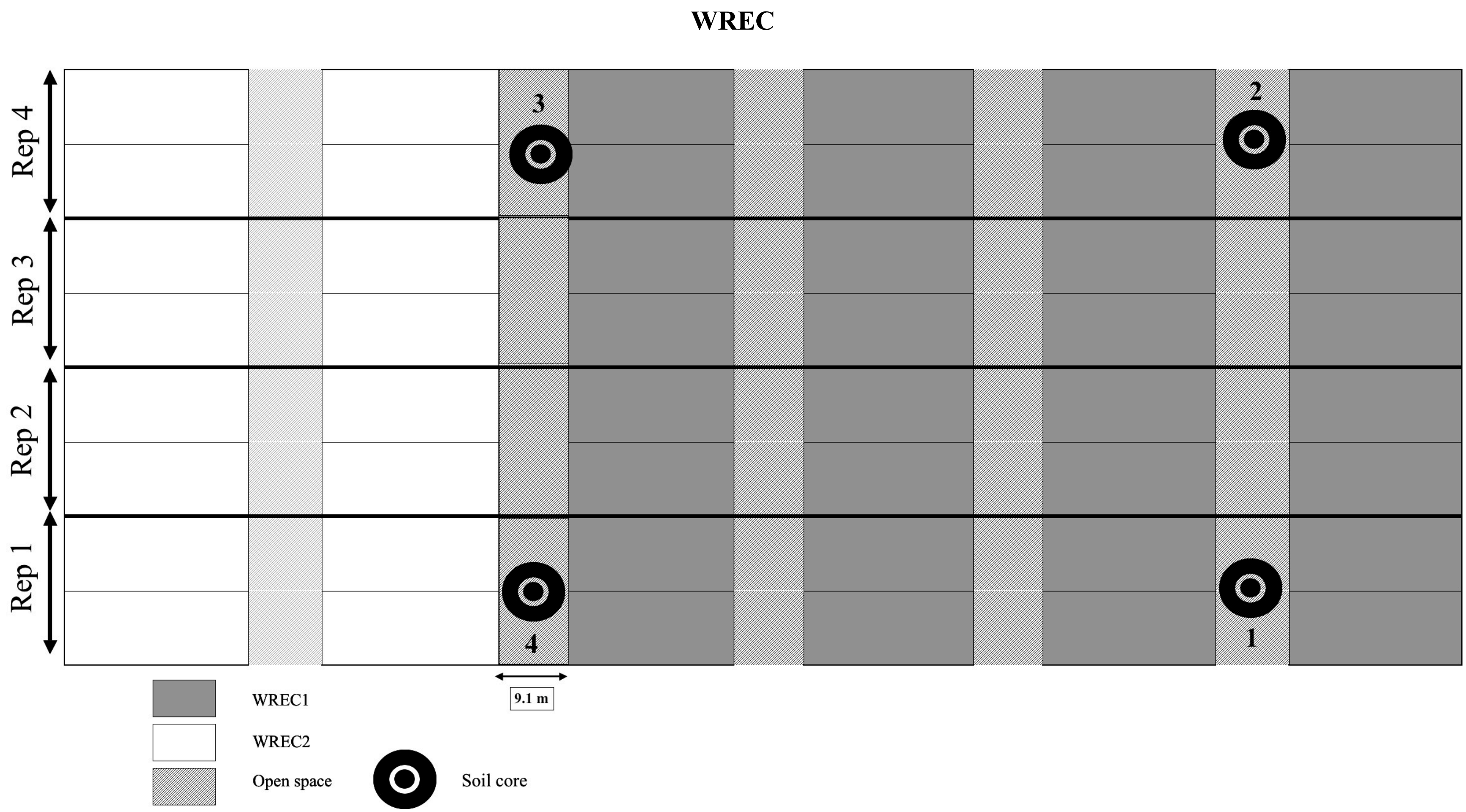
CMREC1



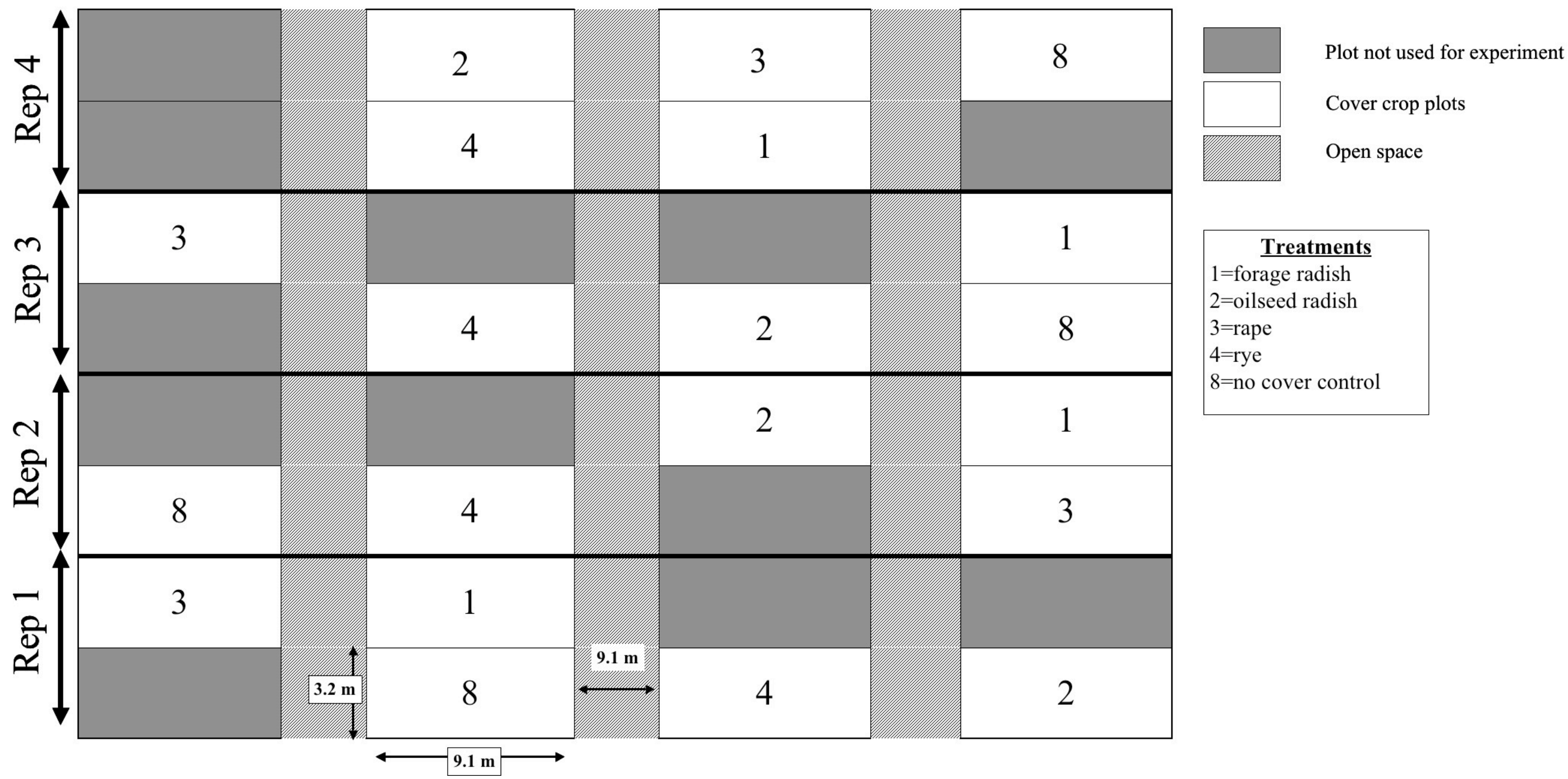
CMREC2



9.1 m
No Cover
Rape
Oilseed Radish
Forage Radish
Rye
Block A
3.7 m
Forage Radish
Rye
Rape
Oilseed Radish
No Cover
Block B
9.1 m
9.1 m
Rye
Forage Radish
No Cover
Oilseed Radish
Rape
Block C
Rye
Oilseed Radish
Rape
No Cover
Forage Radish
Block D
Plot not used for experiment
Cover crop plots
Open space

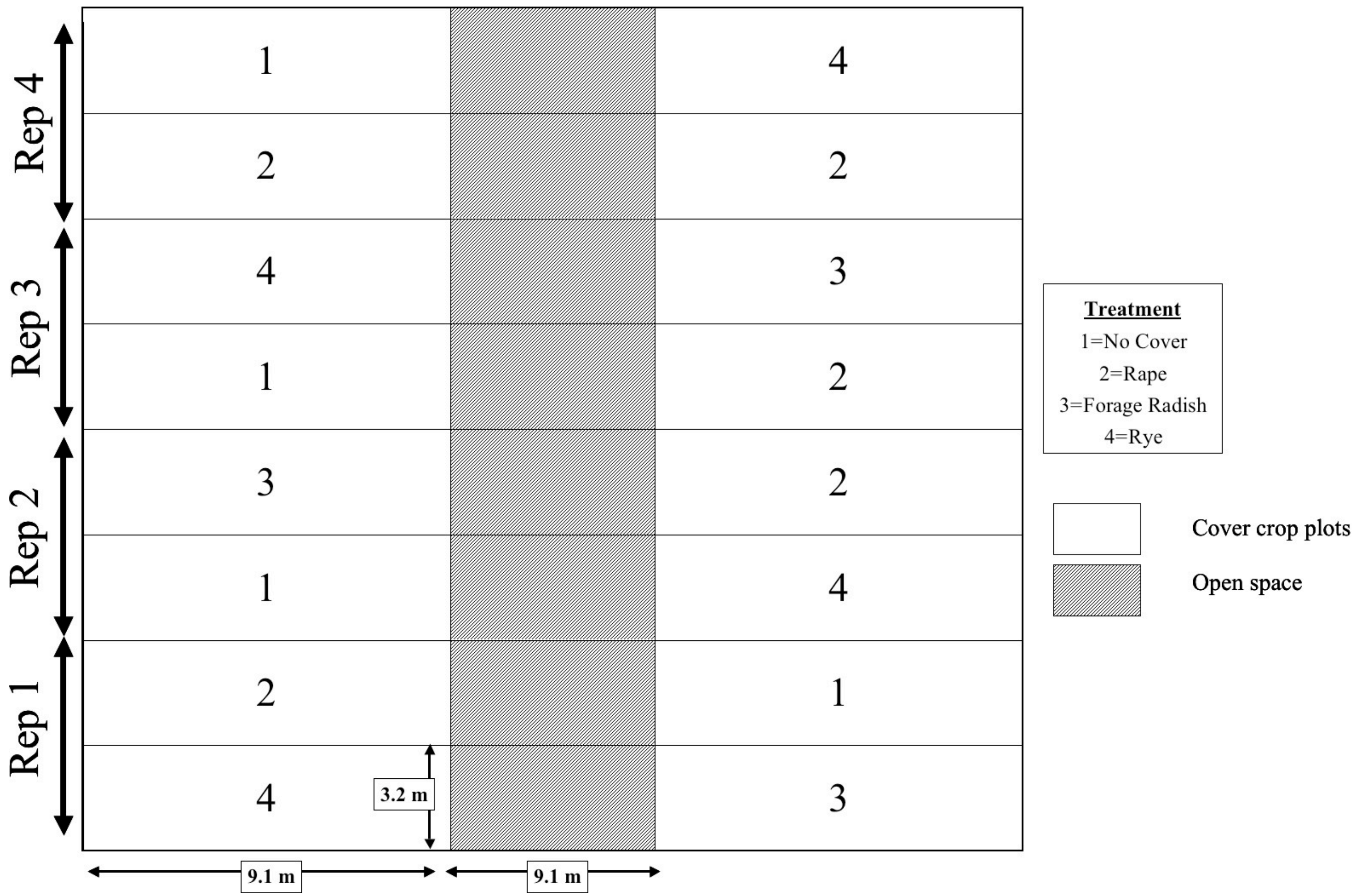

WREC
Rep 4
Rep 3
Rep 2
Rep 1
3
2
4
1
9.1 m
WREC1
WREC2
Open space
Soil core

WREC1



Rep 4
Rep 3
Rep 2
Rep 1
2
3
8
4
1
3
1
4
2
8
2
1
8
4
3
3
1
3.2 m
9.1 m
8
4
2
9.1 m
Plot not used for experiment
Cover crop plots
Open space
Treatments
1=forage radish
2=oilseed radish
3=rape
4=rye
8=no cover control

WREC2



Rep 4
Rep 3
Rep 2
Rep 1
1
4
2
2
4
3
1
2
3
2
1
4
2
1
4
3
3.2 m
9.1 m
9.1 m
Treatment
1=No Cover
2=Rape
3=Forage Radish
4=Rye
Cover crop plots
Open space

Appendix C: Sample SAS Software Commands for Statistical Analysis

A. ANOVA comparing analytes at each 15 cm depth increment of soil

Proc mixed;
By depth season year;
Class rep trt;
Model analyte=trt;
Random rep;
Lsmeans trt/pdiff adjust=tukey;
Quit;

Where "rep" is the block in the experimental design and treated as a random effect, "trt" is the treatment effect, "analyte" is the nutrient to be compared, e.g. NO_3^--N, NH_4^+-N, SON. The results of the ANOVA are separated according to the depth increment the season and year sampled. Least squares means ("lsmeans") are calculated for the treatment effects and pairwise differences between treatments means were calculated using Tukey multiple means comparison test.

B. ANOVA comparing analytes over the entire soil profile sampled (105-180 cm)

Proc mixed;
By season year;
Class rep trt;
Model analyte_in_profile=trt;
Random rep;
Lsmeans trt/pdiff adjust=tukey;
Quit;

Where "rep" is the block in the experimental design and a random effect, "trt" is the treatment effect, "analyte_in_profile" is the nutrient to be compared in the soil profile. The results are separated according to the season and year sampled.

C. Repeated measures ANOVA comparing soil porewater samples

Average over the sampling season:
Proc mixed;
Class time rep subsample trt;
Model analyte=trt;
*Repeated time / sub=rep*trt*subsample type=cs;*
Lsmeans trt/pdiff adjust=tukey;
Quit;

Average for each sampling date:

```
Proc mixed;
By time;
Class time rep subsample trt;
Model analyte=trt;
Repeated time / sub=rep*trt*subsample type=cs;
Lsmeans trt/pdiff adjust=tukey;
Quit;
```

Where “rep” is the block in the experimental design, “time” is the number designation corresponding with weekly sampling and the repeated measure, “subsample” corresponds to one of the two lysimeters within the treatment plot, “trt” is the treatment effect, “analyte” is the nutrient to be compared, “type=cs” is the designated compound symmetry covariance structured.

D. ANOVA for variables associated with plant measurements

```
Proc mixed;
By tissue season year;
Class trt rep;
Model var=trt;
Random rep;
Lsmeans var/pdiff adjust=tukey;
Quit;
```

Where “tissue” is either shoot or root tissue, “trt” is the treatment effect, “rep” is the block of the experimental design and a random effect, “var” is the variable being compared such as biomass or N uptake. Results are separated by the type of tissue and when the plants were sampled.

E. ANOVA for variables across sites and years

```
Proc mixed;
By var1;
Class trt rep site season year;
Model var2=trt|site|season|year;
Random rep rep*trt rep*trt*site rep*trt*site*season;
Lsmeans var2/pdiff adjust=tukey;
Quit;
```

Where “var1” is the variable to sort the results (not necessary), “trt” is the treatment effect, “rep” is the block of the experimental design, “site” is the location of the experiment, “season” is the season during which the samples were taken, “year’ is the year the samples were obtained, “var2” is the dependent variable.

F. Contrasts

Orthogonal contrasts were performed for plant, soil and porewater samples when appropriate. Contrasts for fall-sampled variables differed slightly from spring-sampled variables due to the differences in winter-hardiness of the crops. The following codes are samples of contrasts that would be added into ANOVA codes and are based on the treatments control (C), forage radish (FR), oilseed radish (OR), rape (RA), and rye (RY).

Fall contrast:

Contrast 'mean crops vs. mean control' intercept 0 trt -4 1 1 1 1;
Contrast 'mean brassicas vs. mean rye' intercept 0 trt 0 -1 -1 -1 3;

The matrix for the fall contrasts

-4 1 1 1 1 = 0	-4(0) + 1(-1) + 1(-1) + 1(-1) + 1(3) =
0 -1 -1 -1 3 = 0	0 + -1 + -1 + -1 + 3 = 0

Spring contrast:

Contrast 'mean crops vs. mean control' intercept 0 trt 4 -1 -1 -1 -1;
Contrast 'mean radishes vs. mean rape, rye' intercept 0 1 1 -1 -1;

The matrix for the spring contrasts

4 -1 -1 -1 -1 = 0	4(0) + -1(1) + -1(1) + -1(-1) + -1(-1) =
0 1 1 -1 -1 = 0	0 + -1 + -1 + 1 + 1 = 0

The contrast would be inserted in ANOVA code as follows:

Proc mixed;
By season year;
Class rep trt;
Model analyte_in_profile=trt;
Random rep;
Lsmeans trt/pdiff adjust=tukey;
Contrast 'mean crops vs. mean control' intercept 0 trt 4 -1 -1 -1 -1;
Contrast 'mean radishes vs. mean rape, rye' intercept 0 1 1 -1 -1;
Quit;

G. Testing the Assumptions of ANOVA

Three assumptions must be met before correctly performing ANOVA:

1. experimental units are independent of one another
2. normal distribution of data
3. homogenous variance of data

The first assumption is typically ensured with proper experimental design. The second and third assumptions may be tested using SAS. To test homogeneity of variance, Levene's test is employed (*hovtest=levene*).

```
proc glm;
class trt rep;
model var_=trt;
output out=resids p=yhat r=resid;
means trt / hovtest=levene (type=abs);
quit;
```

Once the residuals of the dependent variable have been calculated and Levene's test has been used, the plot of the residuals can be created (code follows) and the residuals can be examined for both normality and homogeneity of variance.

```
proc plot data=resids;
Title 'Residual Plots for Plant N Data';
plot resid*(trt yhat) / vref=0;
quit;
```

The results of Levene's test are used to confirm the visual inspection of the residuals.

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