ABSTRACT

Title of Document: ***Nitrogen Mineralization from Brassica Cover Crops***

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The potential of forage radish (*Raphanus sativus* L.), rape (*Brassica napus* L.), and rye (*Secale cereale* L.) cover crops to capture residual nitrogen and then provide early season N to subsequent main crops via mineralization from their residues was compared. At four field experiments established in Maryland (2003-2005), N uptake by radish and rape equaled or exceeded that by rye. No differences in soil inorganic N due to cover crop type were observed during spring 2004. In spring 2005, greatest N release from forage radish residues (March-May) was followed by that from rape residues (May-June). Brassica decay significantly increased growth of immature corn and soybean plants. In a 48-day incubation study comparing N mineralization in fine and coarse textured soils from Brassica and rye root or shoot residues, N mineralization was greatest from forage radish and rape shoots. Compared with rye, the Brassica cover crops showed environmental and agronomic promise.

NITROGEN MINERALIZATION FROM BRASSICA COVER CROPS

By

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Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Master of Science
2006

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Acknowledgements

Thanks to: Dr. Weil for the opportunity to be his student; farmers for providing inspiration, my committee for their guidance; UMD-REC farm managers for their patience and assistance; Stan Schlosnagle for cheerfully sharing his lab expertise; the Weil student group, for giving me a chance to laugh every day; and to my husband Boz Wing and my parents Judith and Jeffrey Kremen, for their encouragement and support throughout this experience.

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Chapter 1: Introduction

Background and problem definition

Certain cover crops can be used to improve nutrient cycling in agronomic systems. Some non-leguminous cover crops are highly effective at scavenging nitrogen (N) from soil profiles, reducing N leaching losses in winter when precipitation exceeds evapotranspiration (Meisinger et al., 1991). Winter rye (*Secale cereale* L.), the most commonly planted non-leguminous cover crop in the mid-Atlantic region, scavenges N well but often fails to turn over N stored in its residues in time for use by subsequent summer-season crops, because decomposition of its high C/N ratio residues promotes N immobilization (Wagger, 1989). Identifying other cover crops that are as equally effective as rye at taking up N yet better able to recycle N to subsequent main crops could allow farmers to more precisely determine appropriate levels of manure or fertilizer additions needed on their farms (Shipley et al., 1992; Thorup-Kristensen, 2003).

Justification for research

This research compares the N cycling capacity in the mid-Atlantic of two species in the family Brassicaceae —forage radish (*Raphanus sativus* L.) and rape (*Brassica napus* L.)—to rye. At present, the ability of these Brassicas to take up N and then provide N to subsequent crops in this region is unknown. Based on their performance elsewhere and on preliminary results of the comprehensive research project of which this thesis represents a part, Brassicas have recently been included in a State government program (the Maryland Agricultural Water Quality Cost-Share program, 2006) that offers financial support to farmers who plant cover crops in fall

to reduce winter nutrient leaching within the Chesapeake Bay watershed. The research was intended in part to further evaluate the performance of forage radish and rape with regard to N scavenging and retention from fall through early spring in the Mid-Atlantic region. In addition, this research also attempts to elucidate the timing and quantity of N release in soils from forage radish and rape cover crop residues in spring, information that is essential for effective nutrient management planning. Most published research on N cycling by forage radish and rape is from Denmark and the Netherlands, within farming systems that use conventional tillage (Vos and van der Putten 1997; Vos and van der Putten, 2001; Thorup-Kristensen 1994). The research for this thesis identifies temporal trends in N cycling from the Brassica cover crops in agronomic settings using either no-till management or conventional tillage. The research also investigates N mineralization from root as well as shoot tissues of forage radish and rape shoot tissues, since little information is available on Brassica root tissue decomposition and N release.

General research approach

Replicated field experiments were established at four research sites in Maryland using cover crop treatments of rye, forage radish, rape, and a no-cover control. Total dry matter, N and C/N ratios of cover crop materials (roots and shoots) sampled in late fall and again in spring (for over-wintering species) were measured. Changes in surface soil mineral N (NO_3-N and NH_4-N) during cover crop decomposition were monitored for two growing seasons at 0-15 cm and 15-30 cm depths. Soil was sampled approximately monthly at each site, from March through June during

the first season of study, and from November through August in the second season of study. The Brassica cover crop species selected enabled comparison of N mineralization from forage radish, which winter kills upon hard frosts in the mid-Atlantic region (in December-January) to rape and rye, which over-winter and are killed using chemical or mechanical methods in April. The four field sites provided a range of in-field conditions that can affect N mineralization (including soil texture and bulk density, mean soil and air temperatures, and soil water content).

A 48-day lab incubation was also conducted to compare carbon (C) and N mineralization rates in two soil types (a silt loam and a loamy sand) amended with tissues of forage radish and rape (roots or shoots), and rye (shoots only) under controlled soil moisture (60% water filled pore space) and temperature (25 °C).

General research objectives and hypotheses

The objectives of this research were:

(1) to quantify the N uptake capacity of roots and shoots of an over-wintering Brassica (rape) and one that is killed by winter temperatures (forage radish);
(2) to determine if N is retained by cover crop residues from fall to early spring;
(3) to compare temporal patterns of N mineralization in soil from the different cover crop residues from late winter through mid-spring;
(4) to compare changes in soil inorganic N from forage radish planted alone to a forage radish and rye mixture (this objective was only studied in the first of two field seasons of data collection;
(5) to measure early season response to cover crops in growth and N uptake by corn (*Zea mays*, L.) and soybean (*Glycine max*, L.); and

(6) to compare C and N mineralization in soils (fine textured and coarse textured) amended with root and shoot residues of forage radish, rape, and rye (incubation study).

The hypotheses of this research were that:

(1) Nitrogen uptake in fall by Brassicas will be greater than or equal to rye.

(2) Nitrogen from winter-killed forage radish and over-wintering rape and rye will remain in surface soil and/or cover crop residues and will be available for uptake by summer crops.

(3) The spring N release rate from Brassicas will be faster than that from rye.

(4) The N-release rate from a forage radish and rye mixture will be slower than that from forage radish planted alone.

(5) The N-release rate from cover crop shoots is faster than that from cover crop roots.

(6) Earliest release of N from Brassica cover crops will lead to greater early growth response by subsequent crops.

Chapter 2: Literature review

Introduction

In the mid-Atlantic region, the practice of planting winter cover crops is being encouraged, because these crops absorb nitrogen (N) while growing in fall and winter, thereby limiting leaching losses that cause eutrophication in the Chesapeake Bay. Nitrogen release to main crops from decomposing winter cover crop residues in spring is dependent on residue composition, management, placement, and cover crop rooting depths (Andersen and Jensen, 2001; Bending et al., 1998; Aulakh et al., 1991; Nicolardot et al., 2001; Vaughn and Evanylo, 1998; Thorup-Kristensen, 1993a) as well as soil management (Wilson and Hargrove, 1986; Drinkwater et al., 2000) and environmental conditions (Dabney et al., 2001). Positive yield effects of cover cropping on subsequent main crop yields partly depends on the N concentration of cover crop residues being higher than that in the pre-existing organic matter pool (Baggs et al., 1996); for positive yield effects, the timing of N mineralization from cover crop residues must also be synchronous with greatest N need of main crops (Thorup-Kristensen, 2003). Understanding spring N mineralization kinetics from cover crop residues is important because consistent, substantial N release from cover crop residues to main crops in spring might enable farmers to precisely adjust fertilizer or manure applications while avoiding either production losses or nutrient excesses (Vos and van der Putten, 2000; Justes et al., 1999).

Rye (*Secale cereale* L.), the most commonly planted winter cover crop in the Mid-Atlantic region, is a highly efficient N-scavenger (McCracken et al., 1994; Kuo

et al., 1997). Rye, however, has a limited capacity to recycle N to main crops because its high C/N ratio (>25) residues can cause N immobilization during spring and summer (Wagger, 1989a, 1989b; Vaughn and Evanylo, 1998). Among other non-leguminous cover crops noted as being effective N scavengers, N turnover from Brassica cover crops is expected to be faster than that from rye in spring, mainly because the C/N ratio of their residues is lower (Dabney et al., 2001; Schomberg et al., 2005). Like the research in this thesis, this literature review will focus on the nitrogen cycling of rye and two Brassicas, forage radish (*Raphanus sativus*, L.) and rape (*Brassica napus* L). Accordingly, information in this literature review is organized into three topics: N uptake capacity of rye, rape, and forage radish in fall; N retention of rye, rape and forage radish through winter and early spring; and spring N release dynamics of rye, rape and forage radish and effects on main crops. This review is mostly limited to research conducted in temperate regions, where planting methods, temporal growth, and nutrient cycling trends for these cover crops are expected to be comparable to those used and observed in the mid-Atlantic region. However, information selected from a broader collection of research on nitrogen cycling and cover cropping is included in the manuscript chapters following this literature review.

Nitrogen uptake capacity of rye, rape and forage radish in fall

The fall N uptake capacity of cover crops depends in part on the depth and extensiveness of their root systems (Vos and van der Putten, 1998). At the soil surface (0 to 0.5 m) the amount of nitrogen taken up between rape, forage radish, and rye has

been reported to be roughly equal (Thorup-Kristensen, 1993; Vos and van der Putten, 1998). By contrast, at depths greater than 0.5 m, forage radish and rape have been identified as having a superior capacity than rye to take up N, mostly as a function of having more long roots that penetrate the subsoil (Thorup-Kristensen 2001). Thorup-Kristensen (2001), comparing root growth rates of forage radish, rape and rye, found that these cover crops (planted at the beginning of August in Denmark) reached a rooting depth of 1 m after 751, 789, and 1001 d °C (degree days), respectively. This growth rate difference means that forage radish and rape have a comparative advantage to take up more N in fall because their root systems quickly explore a larger volume of soil (Thorup-Kristensen, 2001). Variability in crop rooting depth is expected across different soil types and climates and with different planting dates. In France, for example, Justes et al., (1999) observed forage radish rooting to only 62 cm from plants that were planted in September and had ceased growing by frost periods in January. In southeastern England, dry conditions in fall have been observed to stimulate deeper rooting growth in rape (Barraclough, 1989). Published data on the effect of dry conditions on rooting depth of forage radish and rye could not be located, but in general (and contrasting with the observation for rape just mentioned), decreased soil moisture levels are expected to have a negative effect on both rooting depth and root diameters of plants (Taylor et al., 1969).

Nitrogen uptake of forage radish, rape, and rye cover crops is expected to decrease (by as much as 2 kg ha^{-1} day^{-1} in temperate regions) with delays in fall planting date, due to deteriorating growing conditions such as decreasing temperatures and daylight hours (Christian et al., 1992; Vos and van der Putten,

1997). On the other hand, planting Brassicas early (in July) can also decrease fall N uptake, since rape and forage radish planted then are inclined to enter reproductive growth phases, during which biomass accumulation is less substantial than during vegetative growth (Eichler et al., 2004). Nitrogen uptake by rye, rape and forge radish is increased when moderate moisture levels (soils are neither saturated nor dried out) exist during growth (Kuo and Jellum, 2000; Smith et al., 1988; Hegde, 1987).

Increasing the amount of N available to cover crops during fall growth stimulates greater N uptake by forage radish, rape, and rye (Vos and van der Putten, 1997; Jensen et al., .1997; Hocking et al., 1997; Thorup-Kristensen et al., 2003). For example, additions of 260 versus 115 kg ha^{-1} N fertilizer in different experimental years led to nearly twice as much uptake by rye (130 versus 69 kg ha^{-1} from 01 August to mid-November) (Thorup Kristensen, 1993). Similarly, when fertilization levels were 20 or 70 kg ha^{-1}, fall N uptake (end of August to December in the Netherlands) by aerial plant parts plus below ground plant parts to 0.1 m depth increased by rye (from 81 to 123 kg ha^{-1}) and rape (from 95 to 138 kg ha^{-1}), respectively (Vos and van der Putten, 2001). Total N uptake by densely growing, N-limited rape volunteers (2600 plants m^{-2}), was estimated at 28 kg N ha^{-1} (shoots and roots combined), including dead leaves that had fallen on the soil (Justes et al., 1999).

Increasing N supply during growth was shown to increase the amount of dry matter and N in aboveground relative to belowground plant parts more for forage radish and rape than for rye, though estimations were rough since belowground plant parts were only collected to 0.1 m (Vos and van der Putten (1997). In a study where

root material was collected more thoroughly, shoot/root ratios of N uptake in forage radish planted in August and collected at the end of October were determined to be between 5 and 6 (Rogasik et al., 1992). Comparing rape plants grown with fertilizer (270 kg N ha^{-1}) or without fertilizer, N concentration in fertilized rape residues (harvested in July following oilseed harvest) was doubled in stems (0.5 to 0.9 %), pod walls (0.3 to 0.6), and roots (0.5 to 1.2) (Trintrousout, 2000).

To achieve optimum N nutrition, Brassicas have been shown in general to require sufficient sulfur (S) nutrition (Schnug et al., 1993). The N/S ratios in soil and in rape shoots have been shown to be positively and significantly correlated (Lasserre et al., 2000). Brassica S nutrition needs and S uptake capacity exceed those of many other plant species, because S is required for oil and glucosinolate (low-molecular weight volatile sulfur compounds common to Brassicas) production in addition to protein synthesis (Mahler et al., 1993; Asare and Scarisbrick, 1995; Lasserre et al., 2000; Schnug et al., 1993). A 7:1 N/S ratio in soils is a desirable balance of nutrients for growing rape, while N/S ratios ranging from 4:1 to 8:1 have been found to be optimum for growing Brassica species in general (Janzen and Bettany, 1984; Schnug et al., 1993). While published information that focused specifically on the N/S nutrition of forage radish could not be found, research by Eriksen and Thorup-Kristensen (2002) suggests that N/S nutrition is as much or more important for forage radish than rye. Between early August and mid-November, forage radish S uptake capacity to a depth of 1 m (36 kg S ha^{-1}) exceeded that of rape (22 kg S ha^{-1} and Italian ryegrass (*Lolium multiflorum* L.) (8 hg S ha^{-1}). Also somewhat unique in

Brassica nutrition is that Brassicas will assimilate more N as NH_4^+ in addition to N as NO_3^- to satisfy N nutrition needs (Lasserre et al., 2000).

The amount of N taken up by rape and forage radish in fall is generally expected to be greater than or equal to that taken up by rye. Across research trials however, the relative N uptake by forage radish, rape and rye is not always consistent. In the Netherlands, Vos and van der Putten (1997) observed that rape and rye cover crops sown by the end of August contained comparable amounts of N (roughly 175 kg ha^{-1}) in their combined shoots and roots after 50 days (roots harvested only to a depth of 0.1 m). In Denmark, Thorup-Kristensen (1994) observed N uptake by between 01 August and mid-November (aboveground parts only) by forage radish (167 kg ha^{-1}) to be significantly greater than rape (127 kg ha^{-1}); N uptake by both Brassicas in that experiment was significantly greater than that of rye (80 kg ha^{-1}). Though Thorup-Kristensen does not specify, it should be noted that the aboveground parts of forage radish includes a portion of the fleshy taproot, which sometimes grows above the soil surface by as much as ~10 cm. We assume that these root parts are included in Thorup-Kristensen's account of forage radish N uptake. Justes et al., (1999) noted total N uptake of 38 N kg ha^{-1} in forage radish shoots and 9 kg N ha^{-1} in forage radish roots from 09 September to the time that frost periods began in January. This variability in N uptake across experiments can be explained by differences in sampling dates, seeding rates, species varieties used, and differences in soil type, climate, and nutrient availability at the different research sites.

Nitrogen retention by rye, rape and forage radish through winter and early spring

Ideally, winter cover crops planted to capture residual nitrogen following main crops will deplete the soil profile of N and then retain that N in their tissues or residues until it can be efficiently utilized by subsequent crops planted the following spring. In the mid-Atlantic region, forage radish is killed by hard frosts in winter, and decomposition begins upon frost-kill. Rape and rye typically over-winter and are killed using mechanical or chemical methods in spring. In an incubation study, both mineralization and nitrification from forage radish residues were observed to be substantial (up to 70% of N mineralized from shoot residues in 140 days), when soil temperatures were kept at 3 °C (Magid et al., 2004). This amount of mineralization was especially noteworthy because the lignin content (usually thought to slow mineralization rates) of these residues was determined to be relatively high (10-11%) (Magid et al., 2004). In a field study in Denmark, Thorup-Kristensen (1994) found that 70% of N in decomposing forage radish residues (aboveground plant parts) had mineralized between fall (mid-November) and spring (late March). While N mineralization from frost killed radish seems to be rapid, research comparing kill method of white mustard (Brassica hirta L.)—by fall incorporation versus allowing frost killed white mustard residues to remain unincorporated—indicates that the latter approach results in relatively slower decomposition and N mineralization through winter into spring (Weinert et al., 2002)

Over-wintering rape and rye can also lose substantial amounts (up to ~100 kg ha^{-1} for rape and ~50 kg ha^{-1} for rye) of the N they took up in fall, when leaves abscise due to shading effects, frost burn, N deficiency, foliar diseases, or senesce

when plants enter reproductive stages of growth (Vos and van der Putten, 1997; Dejoux et al. 2000; Schoerring et al., 1995; Hocking et al., 1997; Thorup-Kristensen, 1994; Colenne et al., 1998). Most reports, however, of N loss from over-wintering cover crops are not so extreme. Typically, little to no change or an increase in N content of over-wintering rye and rape is also possible (Thorup-Kristensen, 1994). Variability in accounts of N loss from cover crops (of rape, in particular) can be explained as being due to differences in N concentrations of cover crop residues, cover crop maturity when sampled in spring, and differences in the severity of winter climate between different experiments. Compared to other plant families, Brassicas have been noted to senesce leaves containing lowest N concentration primarily, and to have a special capacity to translocate N from dying leaves to living plant tissues (Colenne et al., 1998).

N uptake by rape and rye slows as it enters reproductive growth phases in mid-spring (Bhat et al., 1979; Dreccer et al., 2000; Clark et al., 1997). Therefore, N concentration (and C/N ratios) of rape and rye tissues added to soils depends on their kill date in spring (Vos and van der Putten, 1997; Malagoli, et al. 2005; 2005; Hocking et al., 1997; Clark et al., 1997). For example, if let to grow to full maturity for oilseed harvest in July, C/N ratios of rape roots and shoots are well above 50 (Soon and Arshad, 2002; Singh et al., 2006). Ranges for N uptake and C/N ratios of residues (aboveground plant parts only) of rye and rape (measured in March and April, when these covers would be killed in the mid-Atlantic region) are ~32-100 kg ha^{-1} (C/N of ~9-32) and ~40-160 kg ha^{-1} (C/N of ~10-28), respectively (Clark et al., 1997; Kuo et al., 1996; Vos and van der Putten, 2001; Thorup-Kristensen, 1994.).

Roots of rye planted at the beginning of October and harvested 24 April in Washington State had an N content of ~8 kg ha^{-1} and a C/N ratio of ~63 (Kuo et al., 1996). Published information on the N content and C/N ratios of rape roots harvested in March or April could not be found.

Spring N release dynamics of rye, rape, and forage radish and effects on main crops

Turnover of N from additions of plant materials to soils in spring depends on the immobilization-mineralization equilibrium of the soil organic matter pool (Jensen et al., 1997; Thorup-Kristensen, 1993). Justes et al., (1999) observed that rape materials of varying N concentration (including low C/N ratios) were rapidly and equally immobilized following incorporation in an N-limited soil. Extended periods (>5 months) of immobilization in non N-limited soils is expected to be restricted to residues with C/N ratios >24 (Trintrousot et al, 2000b).

Generally, earliest N mineralization is expected from forage radish, rape, and rye shoot tissues since their shoot C/N ratios are lower than their root C/N ratios (Wagger, 1989a). Published information comparing N mineralization rates from shoots and roots of forage radish, and rape residues of maturity levels expected upon killing in the mid-Atlantic region could not be found. Snapp and Borden (2005) reported C/N ratios of over-wintered rye grown in Michigan of 26 and 100 for rye shoots and roots, respectively. Data from a 17-week aerobic incubation where total N turnover from shoots of a white mustard (*Sinapis alba*, L.) green manure significantly exceeded that from roots, and where N release rates were observed to be initially (for the first three weeks of the incubation) higher from shoots than from roots (Chaves et

al., 2004). Kuo et al. (1996) determined that C/N ratios of rape and rye shoots (planted in October) were not significantly different (28 and 33, respectively) in April; therefore the timing of N mineralization in spring from these residues would be expected to be about the same. In the same experiment, the C/N ratio of rape roots (76) was significantly greater than that of rye roots (63), and therefore expectations would be that N release from rape roots would be slower than from rye roots in spring. Freezing and drying of forage radish residues might increase N mineralization from forage radish roots relative to shoots, compared to mineralization from shoots and roots of cover crops killed in spring using mechanical or chemical means (Miller et al., 1994). Within tissue type (shoot or root), faster mineralization rates are expected from tissues with higher N concentrations, especially if soils are non-N limited (rich in C) following incorporation (Justes et al., 1999; Vos and van der Putten, 2001).

While published information on the effect of tillage intensity on release of N from forage radish, rape, and rye in spring could not be located, observations made by Drinkwater et al., (2000) that N mineralization from a hairy vetch (*Vicia villosa*, L.) green manure increased with the level of tillage intensity involved in its management are expected to be similar for other cover crops. In no-till systems, mulch from cover crops is expected to reduce mineralization in early spring because surface soil temperatures will be lower and slower to rise than surface soils of non-mulched (bare) areas (Dabney et al., 2001). Meanwhile, cover crop management by mowing or by killing with glyphosate could affect nitrification; Snapp and Borden (2005)

determined that soil nitrate concentrations in pre-treated (mowed or sprayed) rye residues from mid-May to early June were greater than that from untreated residue.

Two unique chemical qualities of Brassicas should be noted with regard to their potential impact on N turnover from their residues. First, glucosinolates and other organic sulfur compounds common in Brassica residues may be capable of inhibiting nitrification, at least temporarily (days to weeks) (Bending and Lincoln, 2000). This is because various sulfur compounds can act as a substrate for ammonia monooxygenase (AMO, the enzyme that initiates the nitrification process by oxidizing ammonia to hydroxyalamine) and hydroxyalamine ammonia oxidase (HAO) the enzyme that initiates oxidation of hydroxyalamine to nitrite) (Juliette et al., 1993; Bending and Lincoln, 1999; Bending and Lincoln, 2000). The extent to which S compounds might be expected to affect N mineralization from forage radish and rape residues in soils is not known; research data indicate that the content and bio-toxic effect of glucosinolates in Brassicas depends on plant maturity upon killing and the extent of cell wall rupture caused during freezing, crushing, or decomposition of plant tissues (Chen and Andreasson, 2001; Eberlein, et al., 1998; Vaughn and Boydston et al., 1997). Second, plants in the Brassica family are noted for achieving high Calcium (Ca) concentrations during growth (White and Broadley, 2003). For example, Barraclough (1989) noted shoot uptake of rape shoots between planting in August and harvest in July of 290 kg Ca ha^{-1} (plants were field grown in a deep, flinty loam, Charity series). Published data about the effect of increasing surface layer Ca concentration through the deposition of cover crop plant residues in agronomic settings could not be found. In forest ecosystems, however, a Ca increase in surface

soils under in certain tree species (where litter deposition in kg Ca ha^{-1} is similar to or less than that added to soils from Brassica cover crop biomass) has been observed to elevate the soil pH; increasing alkalinity is often associated with greater microbial biomass and higher rates of litter decomposition, soil respiration and net N mineralization (Reich et al., 2005; Gilliam et al., 2005).

Vos and van der Putten (2001), in comparing their results from a field and lab incubation experiment to others', observed that the proportion of N that becomes available to main crops from cover crops can be highly variable. In their field experiment (located near Waginingen, the Netherlands), it was determined that increases in soil mineral N between 01 May and 31 July were derived from 'native' soil organic pools, rather than from forage radish, rape, or rye residues, in spite of the fact that forage radish was killed by freezing temperatures the previous winter. In their lab experiment, however, the authors ensured non-limiting N conditions and noted that 75% of net mineralization from rape, rye, and forage radish residues occurred in the first 5 weeks of an 84-day incubation (controlled conditions of -10 kPa and 20 C°).

Nitrogen released from cover crops in early spring prior to main crop planting is subject to loss via leaching or denitrification, which can lessen positive yield effect of cover cropping on main crops. Vyn et al., (1999) speculated that N loss via leaching or denitrification explained how, when early spring (May) N release was greatest following oilseed radish (compared to red clover and a no cover control treatments), greatest corn yields actually corresponded to highest soil mineral N concentration present in June (following the red clover cover crop treatment). In

addition, if water-filled pore space in soils remains consistently >60% as soils began to warm in spring, denitrification rates, especially during the initial decomposition of non-homogeneously distributed, low C/N ratio (N-rich) cover crop residues, can be intense (Aulakh et al., 1991; Shelton et al., 2000; Magid et al., 2006). In Denmark, Thorup-Kristensen (1994) reported that barley planted on 01 April took up significantly more N until heading out (in June) following forage radish (74 kg N ha^{-1}) and rape (68 kg N ha^{-1}) compared to rye (50 kg N ha^{-1}). When sampled again in mid-July, total N uptake by barley following forage radish (112 kg N ha^{-1}) was significantly greater than both rape and rye (90 and 80 kg N ha^{-1}, respectively, not significantly different).

Summary of N cycling by the Brassicas and rye and discussion of research gaps.

In the mid-Atlantic region, fall N uptake of forage radish and rape is expected to be similar to or greater than that of rye, from planting in mid-August to when cover crop growth rates slow with the approach of winter. After forage radish kills following hard frost, considerable amounts of N mineralization from its residues can be expected in late winter and early spring. The extent of N loss from over-wintering rape and rye cover crops through late winter and early spring could depend on various factors including shading effects, frost burn, plant N deficiency, and foliar diseases. The N concentration in cover crops depends on N availability during growth and cover crop maturity when killed. If soils are N-limited (C rich) in spring, N mineralized from cover crop residues may become immobilized by the soil microbial biomass during the subsequent growing season. In this case, significant nitrogen

release in soil might occur after the period of greatest N need of summer season crops. If soils are not N-limited, earliest and greatest mineralization and nitrification is expected earliest from low C/N ratio residues (plant shoots) compared to high C/N ratios (plant roots). Peak N mineralization from forage radish residues may occur too early to correspond to greatest N need of some main crops. Nitrogen mineralization and nitrification rates are expected to be affected by climatic factors as well as use of tillage versus no-till management of forage radish, rape, and rye residues. Overall, taking the freezing of forage radish residues into account, earliest and greatest N mineralization in spring is expected to be in the order: forage radish>rape>rye. Mineralization and nitrification from forage radish and rape might be either increased or inhibited, respectively, as a result of the Ca concentration and decomposition of S compounds in their residues.

Some research gaps became apparent while compiling information for this literature review. For example, most research on N cycling from cover crop residues, either in field or lab experiments, uses only plant shoot tissues for analysis. In general, there is little research that compares the differences in mineralization and nitrification from root and shoot tissues of cover crops after they are killed. Information published on N cycling with forage radish and rape grown as cover crops in temperate region appears to be mostly limited to a few research groups in Denmark and the Netherlands. More information about N cycling from rape residues is available than exists for forage radish; much of the N mineralization data available regarding rape residues, however, is for rape grown as an oilseed crop (harvested in July, with high C/N ratio residues), rather than as a cover crop (killed in April, with

relatively much lower C/N residues). Information about nutrient cycling with forage radish and rape residues does not exist for the mid-Atlantic region of the US.

Chapter 3: Nitrogen Uptake and Release by Brassica Cover Crops

Abstract

Cover crops that capture residual nitrogen (N) from the soil may reduce both the need for fertilizer and the loss of N if N mineralization from cover crop residues in spring is synchronous with summer crop N demand. The potential of forage radish (*Raphanus sativus* L.), rape (*Brassica napus* L.), and rye (*Secale cereale* L.) to serve as N catch crops as well as to provide inorganic N to subsequent summer crops through N mineralization from their residues was compared. These cover crops differ with regard to their over-wintering potential in Maryland; forage radish is typically killed by frost in December, while rape and rye continue to grow in spring before they are killed by herbicide or tillage.

Cover crop treatments (rye, forage radish, rape, forage radish and rye mixture and a no cover control) were applied in replicated experiments within a winter cover crop/soybean (*Glycine Max* L.)/winter cover crop/corn (*Zea mays* L.) rotation at Maryland research stations in the falls of 2003 and 2004. Measurements included nitrogen uptake by cover crop and immature main crop biomass, surface soil (0-15 and 15-30 cm depths) mineral (NO_3-N and NH_4-N), and total soluble organic N content, soil water content, and bulk density, and air and soil temperature.

In both study years, fall and spring N uptake by Brassica cover crops equaled or exceeded that of rye. There were no treatment effects on soluble soil organic nitrogen content in either field season. Cover crop treatments had little or no effect on inorganic soil N in spring 2004. In spring 2005, soil mineral N levels were higher

at 0 to 15 cm compared to 15 to 30 cm for most treatments and dates, except following large rainfall events in April which may have depleted nitrate-N from the surface (0-30 cm) soil at sites with coarse-textured soils. From February to April 2005, soil NO_3-N concentrations in forage radish plots were usually higher than those in rape, rye and no cover plots. In May and June 2005, after cover crop termination, soil NO_3-N concentration following rape generally exceeded that following rye. Compared to rye or no cover, Brassica residue decay increased early dry matter and N content of seedling corn or soybean plants at most sites. Nitrogen turnover rate from cover crop residues was in the order: fine textured soil with no-till management <coarse-textured no till soils < coarse-textured soils with conventional tillage.

Introduction

Rye (*Secale cereale* L.), the most commonly planted winter cover crop in the mid-Atlantic region, is typically planted in September or October and killed the following April or May using tillage and/or herbicides. Rye is quite effective in reducing erosion, increasing soil organic matter, and scavenging residual N after main crop harvest. Rye also has some drawbacks as a cover crop. Heavy rye residues may interfere with stand establishment of main crops. Also, as the high C/N ratio (> 30) residues decay, immobilization may reduce N availability to the main crop (Allison, 1966; Clark et al., 1997; Wagger, 1989a, b). While rye-legume cover crop mixtures have been used in this region to lower residue C/N ratios and hasten nitrogen cycling (Clark et al., 1994), legumes are not as effective at scavenging nitrogen and overall N uptake might be compromised by using these mixtures (Ranells and Wagger, 1997;

Shipley et al., 1992). To increase farmer options and avoid some of the negative effects of winter cereal cover crops, it would be useful to identify other non-leguminous cover crops efficient at N scavenging that are also more effective than rye in releasing that accumulated N when main crops need it the following spring.

Several studies have identified that Brassica cover crops are as effective at nitrogen scavenging as rye (Sainju and Singh, 1996; Justes et al., 1999; Vos and van der Putten, 2001; Sieling et al., 1999, Stivers-Young, 1998). Compared to rye, N mineralization from Brassica cover crop residues is expected to be more rapid because their C/N ratios are generally lower than that of rye (Schomberg et al., 2005). The N uptake and N cycling capacity of Brassicas in the mid-Atlantic region is unknown. This research used field experiments in Maryland to compare the N uptake and N cycling capacity of two Brassicas, forage radish (*Raphanus sativus* L.) and rape (*Brassica napus* L.), to rye.

Predictions of future N availability to subsequent main crops from cover crop residues is complicated by differences in plant root and shoot biochemical quality (Bending et al. 1998). Differences in N retention by and N mineralization from the two Brassicas studied in this research are expected because rape over-winters (with some leaves abscising from winter to spring), while forage radish is usually completely killed by freezing temperatures in late December or January. Nitrogen mineralization of Brassica residues may be temporarily affected by the bio-toxic effects of glucosinolates, low-weight molecular organic sulfur compounds common in their residues (Bending and Lincoln, 1999). How Brassica residues are managed is also expected to have an effect on nitrogen mineralization. Faster decomposition is

expected when cover crop residues are thoroughly mixed with the soil using conventional tillage compared to residue decomposition in no-till settings (Doran, 1980). Spring killing methods of mowing or using glyphosate might affect N mineralization rates from cover crop residues (Snapp and Borden, 2005).

The main objectives of the present study were to: (1) quantify the N uptake capacity of an over-wintering Brassica (rape) and one that is killed by winter temperatures (forage radish); (2) determine if N is retained by cover crop residues from fall to early spring; (3) compare temporal patterns of N mineralization from the different cover crop residues; (4) compare N mineralization from forage radish planted alone to a forage radish and rye mixture; and (5) measure early season response to cover crops in growth and N uptake by corn (*Zea mays*, L.) and soybean (*Glycine max*, L.).

Materials and Methods

2003/4 (Experiment 1)[1].
Expt. 1 was planted at 4 field sites in Maryland in fall 2003: University of Maryland-Central Maryland Research and Education Center (CMREC); USDA-Beltsville Agricultural Research Center (BARC); Lower Eastern Shore Research and Education Center (LESREC); and Wye Research and Education Center (WREC) (Table 3.1). Selected soil characteristics are presented in Table 3.2.

[1] Abbreviations: Experiment 1, "Expt. 1"; Experiment 2, "Expt. 2."

Table 3.1. Description of field sites, soils, and cover crop seeding dates.

Facility Name	Location in MD (USA)	Soil series and textural class	Cover crop seeding date	
			2003	2004
BARC	Beltsville: 34°04' N; 72°92'W	Elkton silt loam (fine-silty, mixed, active, mesic Typic Endoaquult)	26 August†	‡
CMREC	Beltsville: 39°13' N; 76°86' W	2 blocks: Evesboro loamy sand (mesic, coated lamellic Quartzipsamments); 2 blocks: Cedartown loamy sand (siliceous, mesic psammentic Hapludults)	13 August	25 August
LESREC	Salisbury: 38°36' N; 75°60' W	Norfolk loamy sand (fine-loamy, kaolinitic, thermic Typic Kandiudults	08 August	27 August
WREC	Queenstown: 39°13' N, 76°86' W	Mattapex silt loam (fine-silty, mixed, active, mesic Aquic Hapludults)	19 August	24 September

† Forage radish + rye mixture planted 10 September.
‡ The BARC site was not used during the second experimental year.

Table 3.2. Selected soil properties for the four experimental locations.

Location	Sand	Clay	Organic matter	pH	Bulk density[†]
	mg g^{-1}				g cm^{-3}
		0-15 cm			
BARC	270	240	22	6.1	1.39
CMREC	780	60	14	5.5	1.45
LESREC	830	50	9	6.4	1.54
WREC	270	180	19	5.9	1.46
		15-30 cm			
BARC	--[‡]	--	17	6.0	1.55
CMREC	--	--	6	5.6	1.65
LESREC	--	--	3	6.3	1.79
WREC	--	--	11	5.9	1.57

[†] **Mean value (g/cm^3) of > 100 separate composite samples (10 cores $sample^{-1}$).**

[‡] **Data not collected.**

Mean air temperature and cumulative rainfall data for each site for both experimental years are presented in Figs. 3.1 and 3.2.

The experimental design was a randomized complete block (4 blocks) for plant sampling and split-plot by depth for soil sampling (depth of 0-15 or 15-30 cm). All sites used no-till management except LESREC, where conventional tillage was used. Plot size was 3 by 9 m, except at BARC, where the plot size was 6 by 30.5 m.

Cover crop seeding dates are presented in Table 3.1. The experimental treatments included forage radish (var. 'Dichon'), and a mixture of forage radish and rye (var. 'Aroostook,'), at all sites. Rye alone was included as a treatment at LESREC and WREC. Rape (var. 'Dwarf Essex') was included as a treatment at all sites but CMREC. A "no cover" control treatment was included in each block at all sites.

2004/5 (Experiment 2).

In fall 2004, Expt. 2 was planted on the same fields but in adjacent blocks not planted previously to cover crops, at three of four sites used in Expt. 1 (Table 3.1). The BARC site was not used again due to poor cover crop stands in fall 2004. The experimental design, plot size, and field management methods were the same as in Expt. 1. Cover crop seeding dates are presented in Table 3.1.

The experimental treatments included the same varieties of forage radish and rape as in Expt. 1 but a different variety "Wheeler" was used for the rye treatment. A "no cover" control treatment was included in each block at all sites.

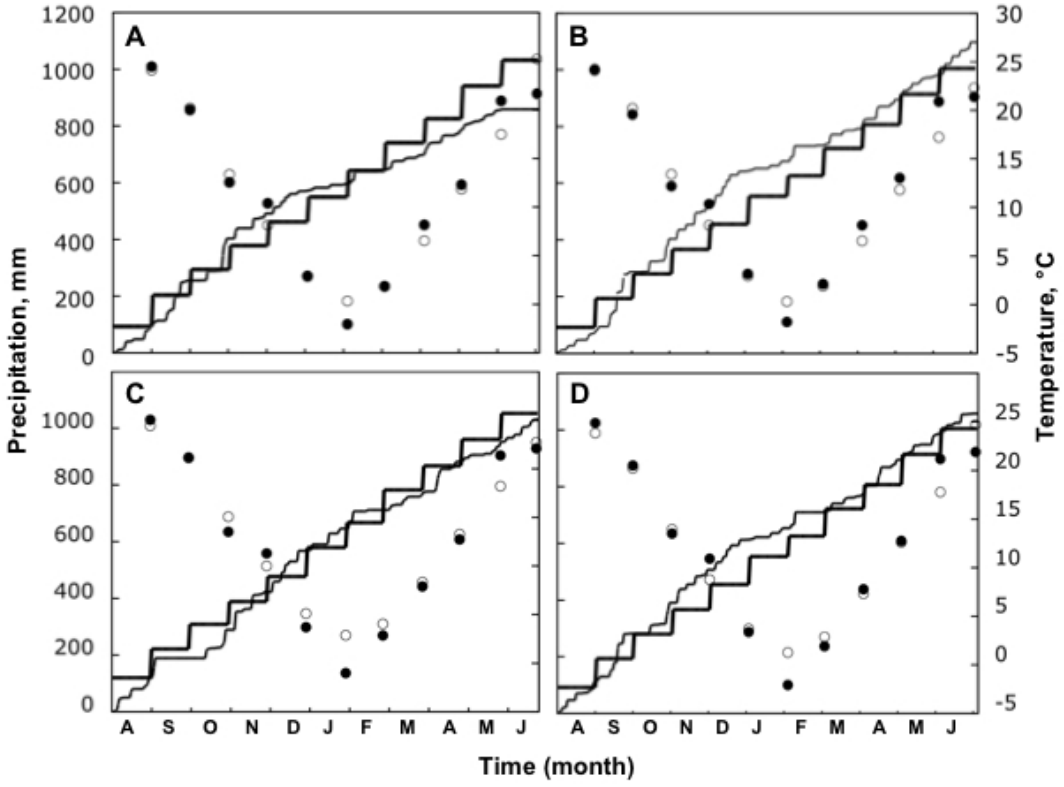

Fig. 3.1. Cumulative daily (—) and cumulative long-term monthly average (——) precipitation, monthly means of daily temperatures during the study (●) and long-term (20 years) monthly average temperature (○), 01 Aug 2003 to 30 June 2004 at BARC (A),CMREC (B), LESREC (C), and WREC (D). LESREC cumulative daily precipitation values include irrigation.

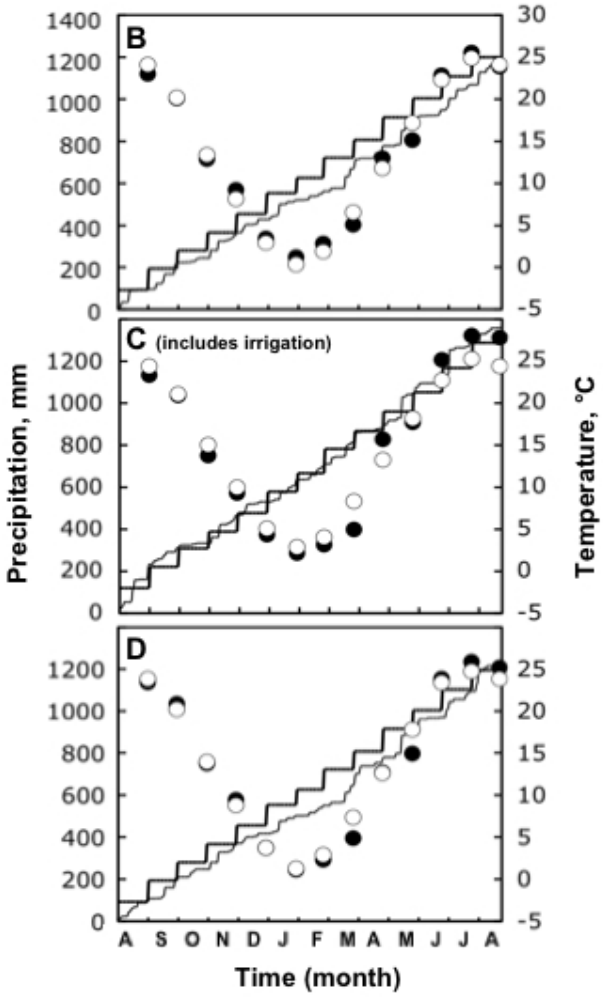

Fig. 3.2. Cumulative daily and cumulative long-term monthly average precipitation, and monthly mean temperature (during the study and long-term average), 01 Aug 2004 to 31 Aug 2005. Refer to Fig. 3.1 for symbol legend.

Site-specific information

BARC (Expt. 1): Two blocks of this field were in fallow (weeds) and two blocks were in potatoes (*Solanum tuberosum* L.) prior to preparing the field to planting the cover crops using a disk and a culti-packer. Cover crops were drill-seeded (1.5 cm depth) with 30.5 cm row spacing without buffers between plots. Freezing temperatures killed forage radish in mid-December, 2003. Rape (100% flowering) and rye (early boot stage) were killed using glyphosate (N-(phosphonomethyl) glycine at a rate of 7 L ha^{-1} (3.41 L ha^{-1} a.i.) on 06 May 2004. Roundup Ready soybeans were drilled on 14 May 2004, with a seeding rate of 224,110 seeds ha^{-1} and 30.5 cm row spacing. Weeds were controlled using glyphosate (1.2 L ha^{-1} a.i.) on 08 June 2004.

CMREC (Expt. 1): Cover crops were first drill-seeded on 13 August 2003 following harvest of a wheat (*Triticum aestivum* L.) crop. Cover crop plots were fertilized 14 August 2003 with 56.0 kg N ha^{-1} as $(NH_4)_2SO_4$ and 134.4 kg ha^{-1} K as K_2O. Forage radish was killed by freezing temperatures in mid-December 2003. Over-wintering rye (boot stage) was killed using 1 L ha^{-1} glyphosate (0.49 L ha^{-1} a.i) on 11 May 2004.

Soybeans (var. NK S39-Q4) were drill-seeded on 12 May 2004 at a rate of at 528,000 seeds ha^{-1} and a row spacing of 76 cm. The field was sprayed with glyphosate (0.49 L ha^{-1} a.i.) for early weed control on 24 June 2004. Mature soybeans were harvested on 17 October, 2004.

CMREC (Expt. 2): To evaluate cover crop N uptake potential on fields with a large amount of mineralizable N, a stand of soybean (R8 stage) was mowed on 18

August 2004, which added ~300 kg ha^{-1} of N to the soil surface (~9000 kg soybean residues ha^{-1} * 0.035% N in residue, as determined by sampling and conducting analysis of multiple 0.25 quadrats of soybean residues that were sampled just prior to mowing). Cover crops were seeded into the soybean residues on 25 August 2004 using a no-till drill. Forage radish was killed by freezing temperatures in the third week of December 2004, and rye (early boot stage) and rape (50% flowering) were killed using glyphosate (2.3 L ha^{-1} a.i.) on 27 April 2005. For routine pH management, 1120 kg ha^{-1} of agricultural lime was broadcast on 05 May 2005. Corn ('Pioneer 34B62') was planted on 10 May 2005 (65,000 seeds ha^{-1} in 76 cm wide rows) without starter fertilizer. Corn was side-dressed by dribbling 112 kg ha^{-1} of N as urea ammonium nitrate solution between corn rows on 15 June 2005. Corn grain and stover was harvested on 12 September 2005. Corn fresh weights were recorded at 26% moisture.

LESREC (Expt 1): Wheat grain (*Triticum aestivum*, L.) was harvested from this site in July prior to beginning the experiment. The field was disked in preparation for planting the cover crops on 07 August 2003. The cover crops were fertilized with 50 kg N ha^{-1} as urea-ammonium nitrate solution on 22 October 2003. Forage radish was killed by freezing in mid-December 2003. All plots were tilled on 28 April 2004 with three passes of a disk harrow with a rear-mounted solid-wheel cultipacker, which killed the living cover crops (rye in boot stage and rape 100% flowering) and incorporated all surface residues. Soybeans (var. NK S39-Q4) were planted on 12 May 2004 (200,000 seeds ha^{-1} in 40 cm rows) and were sprayed with glyphosate (Round-Up 'Gly-4') at 0.38 L ha^{-1} (0.16 L ha^{-1} a.i.) for weed control on 15 June 2004.

Plots were fertilized with 40 kg ha^{-1} N as ammonium nitrate, 50 kg ha^{-1} P as triple super phosphate and 135 kg ha^{-1} K as muriate potash on 29 June 2004. Cyhalothrin-lambda was sprayed on 05 July 2004 at 34.5 g ha^{-1} (4.4 g ha^{-1} a.i.) to control spider mites. The soybeans were harvested on 18 October 2004.

LESREC (Expt.2): Cover crops were drill-seeded 27 August 2004 in an area of the field fallowed the previous year. Shortly after emergence, cover crops were fertilized with 60 kg N ha^{-1} as ammonium sulfate on both 02 September and 22 September 2004. Forage radish was killed by freezing temperatures in the third week of December 2004. Rape (50 % flowering) and rye (boot stage) were killed by mowing on 13 April 2005.

The field was prepared for planting corn by chisel plowing, disking and culti-packing on 13 and 14 April 2005. Corn ('Pioneer 34 B62') was planted on 09 May 2005 at 64,470 seeds ha^{-1} with 76 cm row spacing and was fertilized with 28 kg K ha^{-1} as muriate potash, 100 kg P ha^{-1} as triple super phosphate, 28 kg S ha^{-1} and 1 kg B ha^{-1} as borate. Mature corn was harvested and the grain combined on 25 September 2005.

WREC (Expt. 1): Prior to planting the cover crop experiment, the field site history was no-till management of a corn and soybean rotation, with sweet corn grown in 2003. The cover crops were drill-seeded on 19 August 2003. Forage radish was killed by freezing temperatures in mid-December 2003 and rye (boot stage) and rape (flowering) were killed by rolling and spraying with glyphosate (1.4 L ha^{-1} a.i.) on 05 May 2004. Soybeans (var. NK S39-Q4) were drill-seeded on 21 May 2004 at a seeding rate of 528,000 seeds ha^{-1} with a row spacing of 76 cm. Glyphosate was

sprayed (1.9 L ha^{-1} a.i.) for weed control on 23 June 2004. Soybeans were combined the week of 13 October 2004.

WREC (Expt. 2): Soybeans (R8 stage, same planting date as in Expt. 1) were mowed on 18 September 2004 on an adjacent portion of the same field to establish an area for the 2004/5 experiment, adding ~250 kg ha^{-1} N to the soil surface (7300 kg soybean residues ha^{-1} * 0.035% N). Forage radish was killed by freezing temperatures in the third week of December 2004 and rape (50 % flowering) and rye (early boot stage) were killed by applying glyphosate (1.9 L ha^{-1} a.i.) on 03 May 2005. Corn ('Pioneer' 34B62) was drill-seeded without starter fertilizer on 19 May 2005 at a rate of 40,000 seeds ha^{-1} in 76 cm rows. Mature corn was harvested for dry weight analysis on 29 September 2005.

Plant sampling and analysis

Plant sampling methods were the same for Expts. 1 and 2. All plant sampling dates are presented in Table 3.3. For cover crops, shoot and fleshy portion of the root of rape and forage radish and shoot of rye were sampled in fall by removing two 0.25 m^2 quadrats per plot near the time of maximum growth for the fall season but prior to winter kill of forage radish. Dead and decomposing forage radish residues were not sampled in spring. Roots were washed with distilled water in the field and again in the lab. It is important to note that the juncture of forage radish shoot and root is typically several cm above the soil surface. Because ~20 to 40% of the fleshy radish root typically grows above ground, our references in this paper to "shoot" and "root" dry matter pertains to a separation of plant parts based on physiological differences, rather than parts of plant tissue growing either above or the soil surface.

Plant samples were dried at 60 °C in a forced draft oven for 3+ days for determination of dry matter and then ground and sieved to <1 mm. Sub-samples were prepared for N analysis by LECO CHN-2000 analyzer (LECO Corporation; St. Joseph, Michigan) (Campbell, 1992). To evaluate cover crop effects on immature main crops, soybeans (V1 stage prior to nodule formation) were sampled from randomly selected 2-meter lengths of row in forage radish and no cover control plots within two weeks of planting in May 2004 (Expt.1). In Expt. 2, 16 corn plants (V6 stage) were randomly harvested from each plot (except in the rape plots at CMREC) at all sites in mid-June, 2005.

Soil sampling and analysis

Soil sampling methods were the same for Expts. 1 and 2. Soils were sampled using a ~2 cm diameter soil probe on selected dates in spring in Expt. 1 and from late fall-early winter (November-December) through mid June in Expt. 2 (Table 3.3). Ten soil cores were taken from random locations in each plot; portions of cores from 0-15 cm and 15-30 cm depths were bagged separately to create 2 composite samples per plot. Soils were stored on ice after sampling and brought promptly to the lab for processing. Soil samples were weighed moist, rapidly dried at 60 °C, weighed dry for moisture content and bulk density determination, ground to pass a 2 mm sieve, and stored at room temperature in sealed plastic bags until sub-samples were prepared for analysis. Bulk density was determined by dividing the dry weight of the total sample by ((π * radius of soil core^2 * 15 cm (h)*10 cores per sample).

Table 3.3. Cover crop biomass and soil sampling dates.

Location	Sample type		
	Fall 2003 biomass	Spring 2004 biomass	Soil (2004)
USDA-BARC	15 November	28 April	15 March, 17 May, 17 June
CMREC	5 October-12 October	†	14 March, May 8, June 16
LESREC	18 October	23 April	18 April, 14 June
WREC	22 November	01 May	01 March, 15 May, 19 June
	Fall 2004 biomass	Spring 2005 biomass	Soil (2004-5)
CMREC	30 October	23 April	08 December, 07 January, 11 March, 15 April, 14 May, 11 June, 21 August
LESREC	08 November	13 April	15 November, 09 January, 16 February, 01 April, 06 May, 12 June, 20 August
WREC	11 November	23 April	17 November, 10 January, 17 March, 06 April, 22 May, 20 June, 25 August

†None collected.

Three grams of dried, ground and sieved soil was shaken with 30 ml of 0.5 *M* K_2SO_4 for 30 minutes at 100 rpm, centrifuged for 2 minutes at 3000 rpm, and then filtered (Fisher Q2 filter papers, Fisher Scientific cat. # S47578C). Filtrates were analyzed immediately or refrigerated for no more than 48 hours before analysis.

Soil ammonium-N concentration was determined using an Orion 9512 ammonia specific gas-sensitive electrode (Banwart et al., 1972). Standards (0.1, 1, 10 and100 mg NH_4-N L^{-1}) were prepared with NH_4Cl in 0.5 *M* K_2SO_4. One ml of 5*M* NaOH ionic strength adjusting solution with pH color indicator was added to 10.0 ml of sample to raise sample pH above 13. Ammonium-N concentrations (mg kg^{-1}) in samples were determined by constructing a logarithmic standard curve (log millivolts versus mg L^{-1} of standard) and multiplying by a dilution factor (30 ml extractant used/3 g soil).

Nitrate concentration was determined in filtered samples by using a flow injection analyzer (Technicon Industrial Systems; Tarrytown, NY) with a cadmium reduction column and a 2:1 distilled water dilution loop (Technicon Industrial Method No. 487-77A, 1977).

Total dissolved N (TDN) was determined by using an alkaline persulfate autoclave digestion that oxidized all N in the sample to nitrate (U.S. Geological Survey Analytical Procedure NWQL SOP # 1M0384.0, 2003). We modified the digestion method, designed for water samples, for use with soil extractions using K_2SO_4. Fifteen ml of sample (extract) and 15 ml of alkaline potatssium persulfate reagent (45 g K_2SO_8 and 9.5 g NaOH per 1 L distilled deionized H_2O) was put in 30

ml Borosilicate glass culture tubes (Fisher Scientific cat. # 14 957 76E), which were sealed tightly by hand using screw-top polypropylene linerless caps (Fisher Scientific cat. # 0334077E). Because the digestion process acidified the digestate, 1.5 ml of 0.3 *M* NaOH in 10% sodium acetate was added to each digested sample and standard immediately prior to nitrate measurement to bring the digestate to the pH range optimal for measurement with the Technicon Autoanalyzer II flow injection analyzer (Technicon Industrial Systems; Tarrytown, NY). Total soluble organic nitrogen (TSON) was calculated by subtracting the NO_3-N and NH_4-N values obtained from the same sample material from the TDN value.

For all N analyses, bulk density data specific to each sample were used to convert soil N concentrations in milligrams per kilogram of soil to N mass in kilograms per hectare of soil.

Statistical analysis

Cover crop biomass was subjected to ANOVA for each site and sampling date separately using the MIXED procedure in SAS (SAS Institute, 2005). In this case, the "block" factor in the RCBD was treated as a random factor. Comparisons of fall and spring cover crop biomass size and N uptake were analyzed for each sampling location separately using t-tests, with significant differences determined at $P<0.05$. Mineral N concentration data in soil samples (0-30 cm) was subjected to ANOVA for each location and sampling date separately using the MIXED procedure in SAS. When comparing mineral N concentration in the 0-15 and 15-30 cm layers, the ANOVA was run as a split plot by depth, with the different soil layers identified as a sub-plot factor in the random statement to obtain the correct number of degrees of

freedom for the analysis. The significance of mean comparison differences was estimated by using the Tukey-Kramer adjustment, with $P \leq 0.05$. Repeated measures was not used because of treatment*date interactions.

Results

Cover crop biomass production and N uptake

Fall 2003, 2004. Cover crop dry matter and N uptake data from fall 2003 could not be subjected to analysis of variance due to limited collection of cover crops from an unbalanced number of blocks within each site (Table 3.4). Shoot biomass production and N uptake in fall 2004 (Expt. 2) from Brassicas was comparable to or significantly exceeded that of rye (2-5,000 kg ha^{-1}) (Table 3.5).

In Expt, 2, N concentration of forage radish root dry matter (2.3 mg g^{-1}) exceeded that of rape roots (~ 2.1 mg g^{-1}). Nitrogen uptake by Brassica (root and shoot combined) ranged from 80-250 kg ha^{-1} in Expt. 1 and 100-200 kg ha^{-1} in Expt. 2. Combining fall data for all cover crops at all sites in both years, there was no relationship ($R^2 \leq 0.2739$) between N uptake by cover crops (above or below ground parts) and planting date, except for rape roots, where N uptake decreased with later planting dates. (R^2 =0.8005) (data not presented).

Spring 2004, 2005. Though not enough samples were collected to make a statistical comparison, the dry matter and total N content of rye shoots and rape roots harvested just prior to being killed in spring 2004 appeared to remain constant relative to their levels the previous fall. T-test comparisons across the two sites where rape shoots were collected indicate that rape shoot biomass production and N

uptake remained constant relative to their levels the previous fall. Within each location, t-test comparisons of biomass production and N uptake for spring 2005 indicate that biomass production and N uptake levels of all covers remained constant from fall 2004 to the following spring at CMREC and LESREC. At WREC, spring 2005 cover crop biomass production significantly increased relative to levels observed the previous fall; meanwhile levels of N uptake in spring 2005 at WREC significantly decreased, relative to their levels the previous fall. In spring 2005, rye shoots generally contained less N than rape shoots (significantly only at LESREC even though these cover crops did not differ with respect to the magnitude of their biomass production.

Averaging across both years for all sites (treating location as random), biomass production and N uptake by forage radish roots in fall (3374 kg biomass ha^{-1}, 74 kg N ha^{-1}) significantly exceeded that of rape roots (1500 kg biomass ha^{-1}, 28 kg N ha^{-1}). Also in fall, rape shoot biomass (4556 kg biomass ha^{-1}) exceeded that of rye (2642 kg biomass ha^{-1}). Fall N uptake of rape shoots (138 kg N ha^{-1}) and forage radish shoots (107 kg N ha^{-1}) exceeded that of rye shoots (61 kg N ha^{-1}). In spring, biomass production of rape shoots and rye shoots (4500 kg ha^{-1}) were not different, though N uptake of rape shoots (94 kg N ha^{-1}) exceeded N uptake of rye shoots (68 kg ha^{-1}).

Table 3.4. Cover crop biomass and N uptake, Fall 2003 and Spring 2004.

Location	Cover crop	Plant part	Biomass (dwt)	N uptake	Biomass (dwt)	N uptake	C:N
			Fall 2003 kg ha^{-1}		Spring 2004 kg ha^{-1}		
BARC	Forage radish	root	1457 (557) †	27 (10)	‡	‡	‡
	Rape	shoot	2094 (947)	44 (20)	2385 (336)	21 (3)	32
	Forage radish	shoot	2821 (753)	60 (16)	‡	‡	‡
	Rape	shoot	3122 (1069)	102 (33)	3972 (630)	65 (10)	23
	Rye in F. Radish+	shoot	‡	‡	‡	‡	‡
CMREC	Forage radish	root	1446 (127)	27 (2)	‡	‡	‡
	Forage radish	shoot	4650 (593)	100 (9)	‡	‡	‡
	Rye in F. Radish+	shoot	‡	‡	‡	‡	‡
LESREC	Forage radish	root	1254 (120)	23 (2)	‡	‡	‡
	Rape	root	‡	‡	2827 (509)	25 (5)	27
	Forage radish	shoot	3948 (651)	84 (14)	‡	‡	‡
	Rape	shoot	‡	‡	7317 (1070) a§	117 (13) a	23
	Rye	shoot	‡	‡	5379 (878)	99 (9)	33
	Rye in F. Radish+	shoot	‡	‡	4019 (492) b	70 (8) b	25
WREC	Forage radish	root	8760 (1320)	162 (24)	‡	‡	‡
	Rape	root	3300 (1180)	69 (25)	‡	‡	‡
	Forage radish	shoot	7300 (2404)	156 (36)	‡	‡	‡
	Rape	shoot	8960 (2093)	292 (48)	3124 (867) a	56 (13) a	27
	Rye	shoot	5920 (2263)	148 (54)	2533 (274)	47 (5)	28
	Rye in F. Radish+	shoot		‡	1923 (111) b	34 (2) b	27

† Numbers in parentheses are SEM. In 2003 :BARC (n=2); CMREC (n=4); LESREC (n=4); WREC (n=2); in 2004: n=4, all sites.

‡ Plant biomass was not collected and/or nutrient content was not determined.

§ Within locations and rows, numbers followed by the same letter are not statistically different (P<0.05, using Tukey-Kramer adjustment).

Table 3.5. Cover crop biomass and N uptake, Fall 2004 and Spring, 2005.

Location	Cover crop	Plant part	Biomass (dwt)	N uptake	C/N ratio	Biomass (dwt)	N uptake	C/N ratio
			Fall 2004 kg ha^{-1}			Spring 2005 kg ha^{-1}		
CMREC	Forage radish	root	475(1241)[†] *a*[‡]	146 (29) *a*	13	§	§	§
	Rape	root	1138 (167) *b*	27 (3) *b*	16	1533 (331)	22 (4)	§
	Forage radish	shoot	1943 (218) b	75 (4) ab	10	§	§	§
	Rape	shoot	3009 (201) a	110 (18) a	12	3140 (406)	84 (13)	16
	Rye	shoot	1029 (207) c	41 (9) b	12	4659 (818)	75 (9)	27
LESREC	Forage radish	root	2258 (75) a	46 (6) a	20	§	§	§
	Rape	root	748 (128) b	19 (6) b	18	§	§	§
	Forage radish	shoot	3647 (441) a	161 (24) *a*	9	§	§	§
	Rape	shoot	4123 (406) *a*	119 (31) ab	15	5081 (144)	147 (5) *a*	14
	Rye	shoot	2221 (242) *b*	61 (11) *b*	16	3649 (112)	82 (15) *b*	20
WREC	Forage radish	root	3843 (423) a	61 (6) a	25	§	§	§
	Rape	root	1413 (172) b	21 (2) b	26	2842 (150)	20 (1)	§
	Forage radish	shoot	4343 (220) ab	155 (19) ab	12	§	§	§
	Rape	shoot	**5053** [§] (528) a	**171** [¶] (22) *a*	13	**6466** (415)	**117** (12)	23
	Rye	shoot	**3210** (233) b	**99** (11) *b*	15	**6221** (1549)	**86** (18)	31

[†]Numbers in parentheses are SEM, n=4

[‡]Within plant part type and location, numbers followed by different letters are statistically different ($p<0.05$ or if letters are italicized, $p<0.10$), using Tukey-Kramer adjustment.

[§] Plant biomass was not collected and/or nutrient content not determined.

[¶] Bold numbers for biomass and N uptake highlight comparisons where Spring biomass was statistically greater than Fall biomass, but the N concentration of Spring biomass was statistically less than Fall biomass (using t-test, $p<0.05$).

Soil mineral N

Expt. 1: Ammonium-N in the 0-15 cm and 15-30 cm layers of soils remained at around 5 kg ha^{-1} for all treatments throughout the study period at all sites except at LESREC, where NH_4-N decreased to near zero from about 10 kg ha^{-1} for all treatments from April to June (Figs. 3.4-3.7). Nitrate-N in both soil layers (0-15 cm and 15-30 cm) increased moderately during sampling dates from April to June, from $\leq$ 5 kg ha^{-1} to roughly 10-15 kg ha^{-1} at all sites except CMREC, where NO_3-N remained unchanged in the surface layer and decreased to almost zero in the 15-30 cm layer (Figs. 3.3-3.6). Generally, decreases in soil NH_4-N concentration were synchronous with increases in soil NO_3-N concentration. No treatment effects of cover crops on soil inorganic N concentration were observed for any sampling dates in spring 2004 within each of the two soil layers studied. At all dates, no statistical difference in inorganic N concentration existed for the two soil layers studied.

Combining inorganic N concentrations for both soil layers, the only treatment difference occurred at LESREC in April, where inorganic N (in 0-30 cm of soil) in the forage radish plots significantly exceeded that in the rye and no cover plots (Fig. 3.7).

Expt. 2: On sampling dates prior to hard frost (which occurred in the third week of December at all sites), inorganic N concentration (nitrate in particular) in both soil layers in all cover crop plots was low and unaffected by cover crop (Figs. 3.8-3.10) except at WREC, where NO_3-N concentration in the no cover treatment significantly exceeded that of the other treatments in the 15-30 cm soil layer.

From January through the rest of spring sampling, almost no treatment differences were observed in the ammonium-N fraction of soil inorganic N for all treatments in both soil layers at all sites from January to mid-June. The exception was at LESREC, where NH_4-N levels were significantly higher in the forage radish plots in January and February. On most dates at all sites, NH_4-N in the 0-15 cm layer significantly exceeded that in the 15-30 cm layer. The exceptions were at WREC (April) where NH_4-N in the 15-30 cm layer was greater that in the 0-15 cm layer, and in August at CMREC and WREC, when NH_4-N concentration in the two soil layers studied was not significantly different. Peak soil NH_4-N concentrations (in May) appeared to coincide with or precede (by a month) peaks in soil NO_3-N concentration.

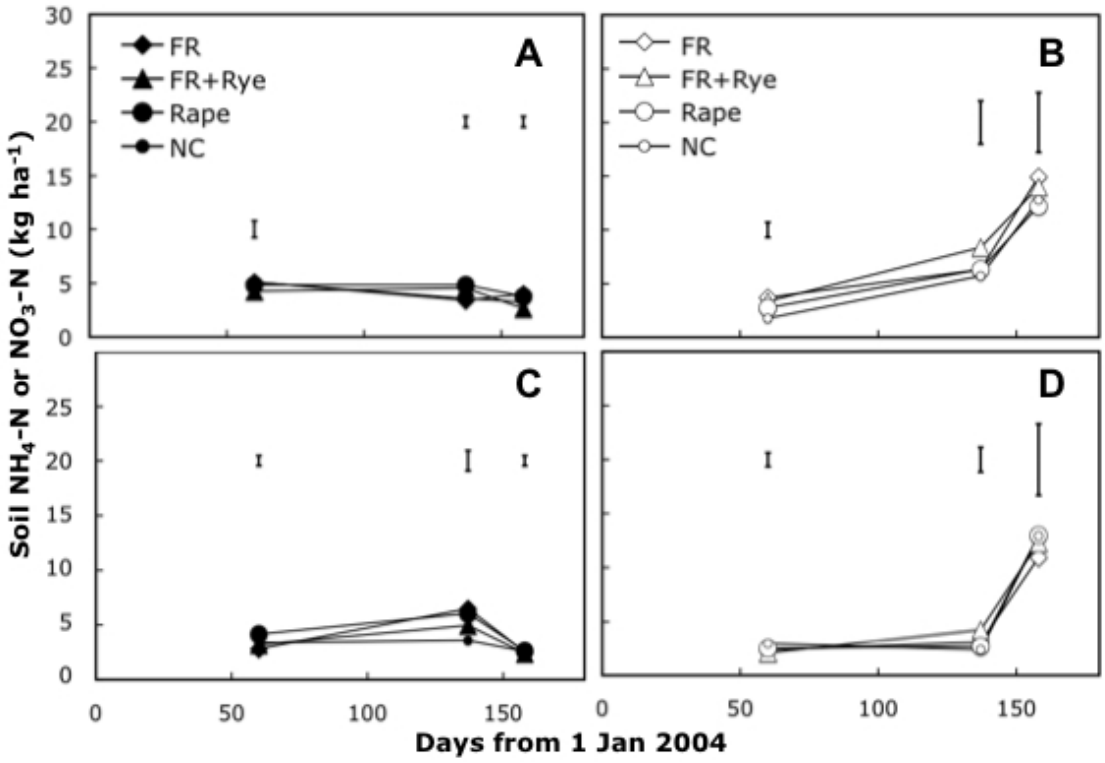

Fig. 3.3. Expt. 1: Ammonium (filled symbols) and nitrate (unfilled symbols) at 0-15 cm (A,B) and 15-30 cm (C,D) depths at BARC. Abbreviations of FR and NC refer to the forage radish and no cover control treatments, respectively. Vertical bars are average SEM.

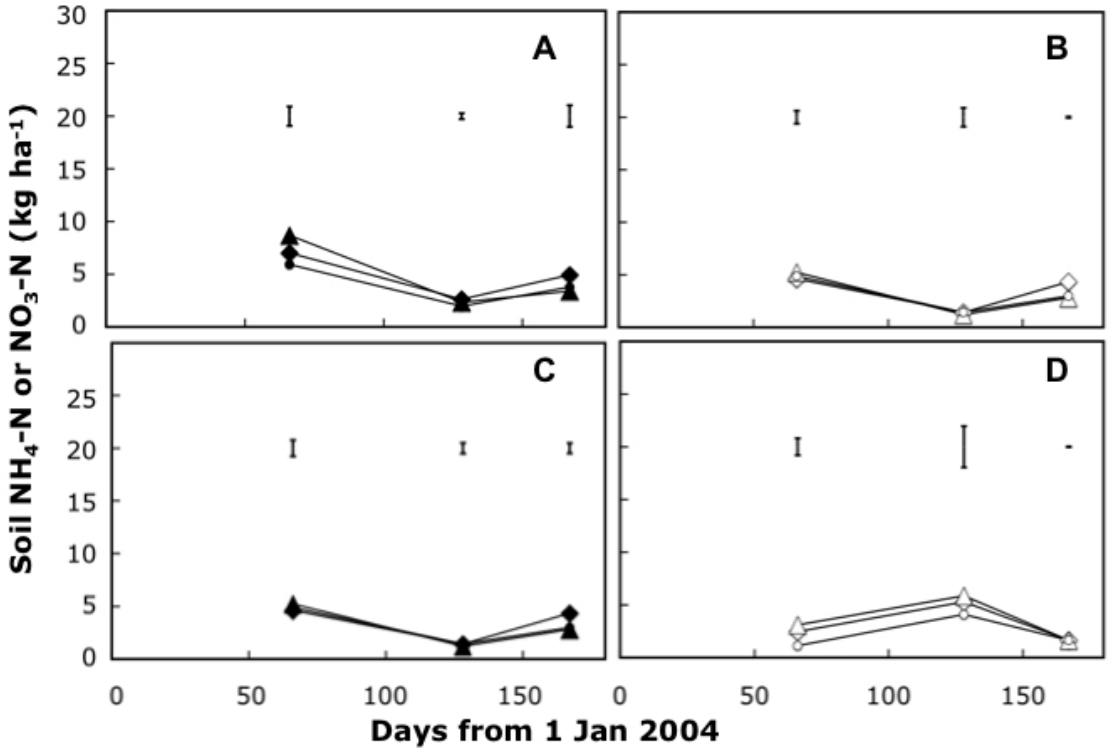

Fig. 3.4. Expt. 1: Ammonium (filled symbols) and nitrate (unfilled symbols) at 0-15 cm (A,B) and 15-30 cm (C,D) depths at CMREC. Refer to Fig. 3.3 for symbols. Vertical bars are average SEM.

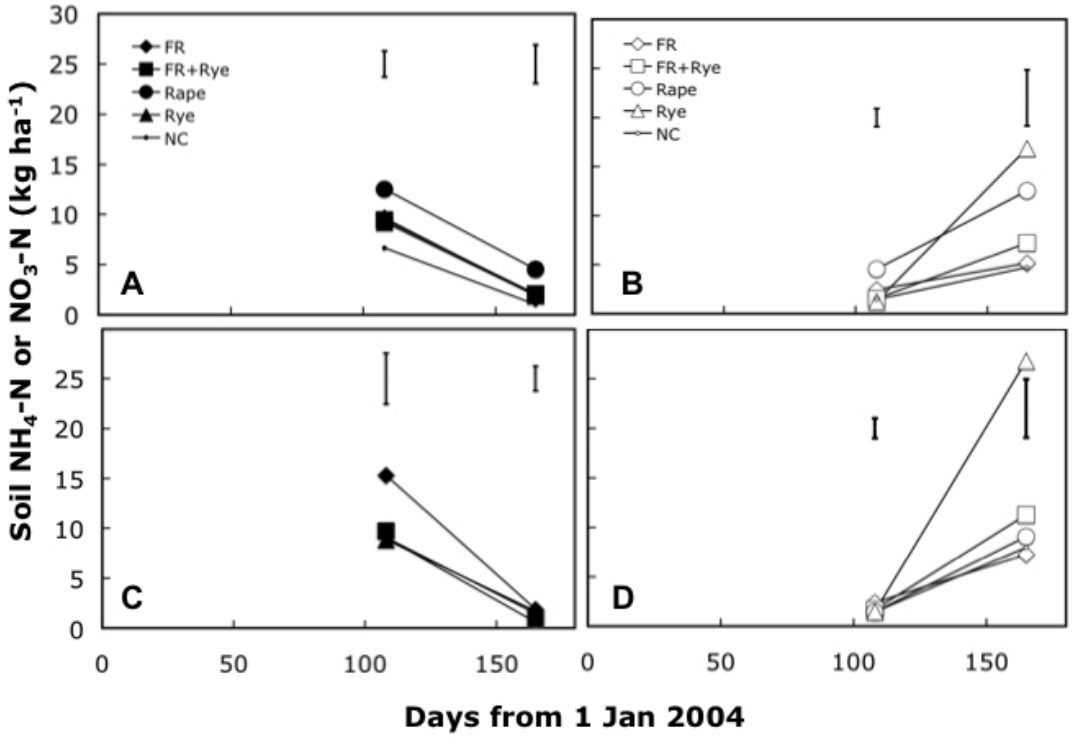

Fig. 3.5. Expt. 1: Ammonium (filled symbols) and nitrate (unfilled symbols) at 0-15 cm (A,B) and 15-30 cm (C,D) depths at LESREC. "FR+Rye" in the symbol legend refers to the forage radish plus rye mixture treatment. Vertical bars are average SEM.

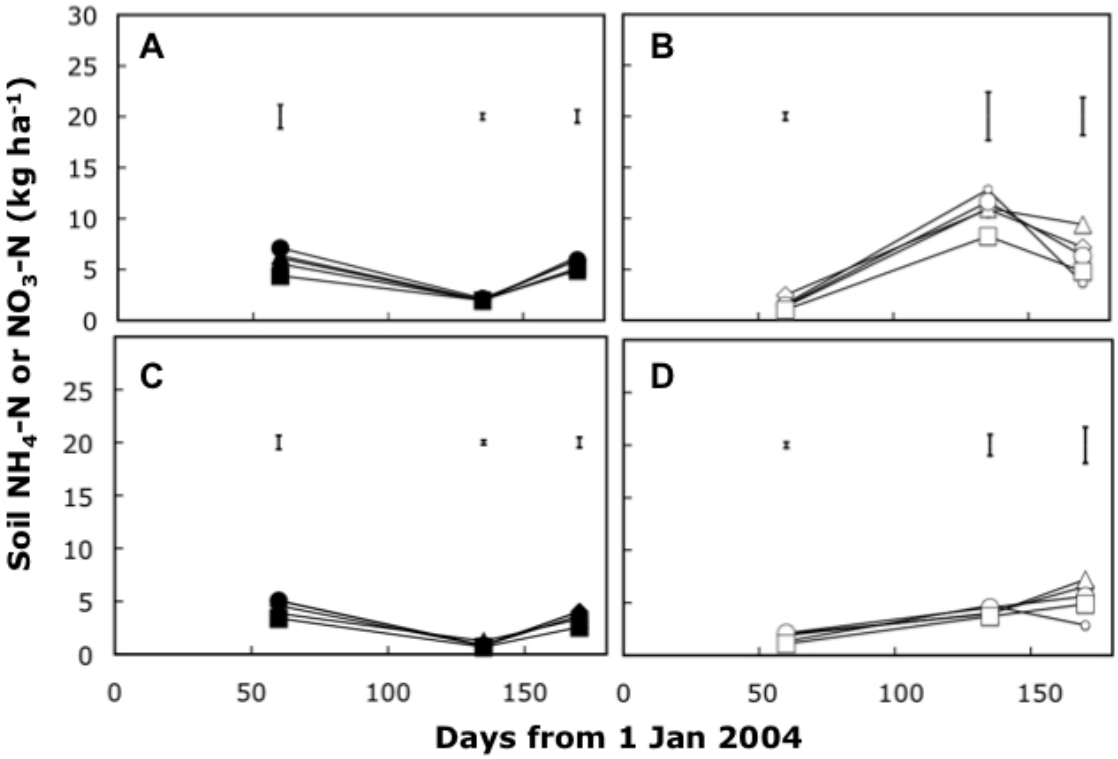

Fig. 3.6. Expt. 1: Ammonium (filled symbols) and nitrate (unfilled symbols) at 0-15 cm (A,B) and 15-30 cm (C,D) depths at WREC. Refer to Fig. 3.5 for symbols. Vertical bars are average SEM.

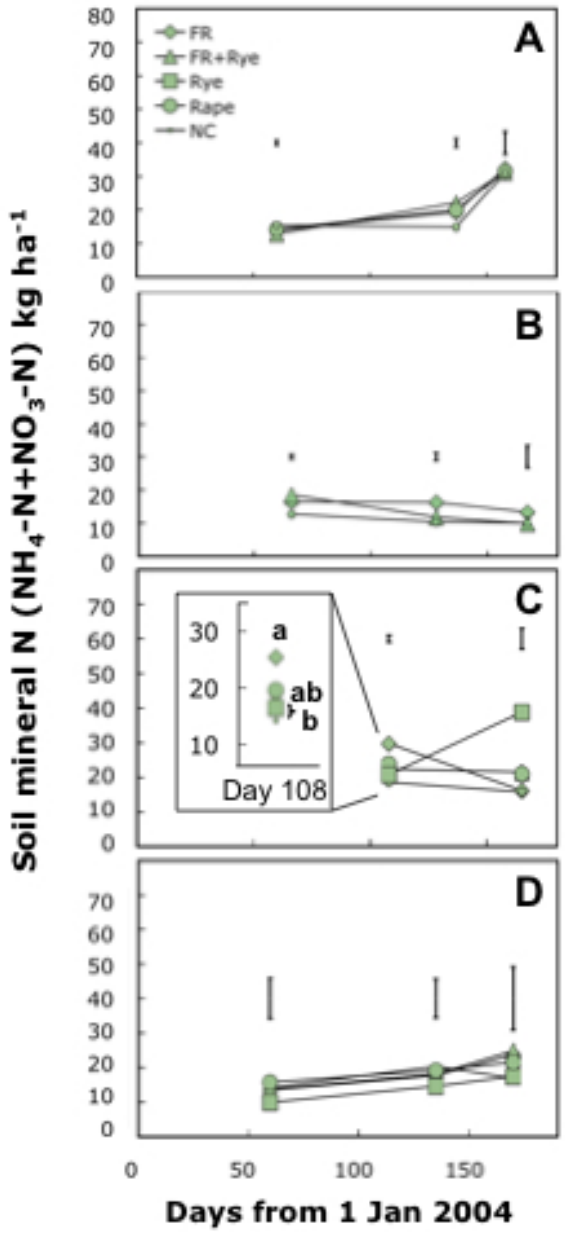

Fig. 3.7. Expt. 1: Soil mineral nitrogen (0-30 cm) at BARC (A) CMREC (B), LESREC (C) and WREC (D). Vertical bars are average SEM. Mean values with the same under case letter are not statistically different at ($P<0.05$), using Tukey-Kramer adjustment.

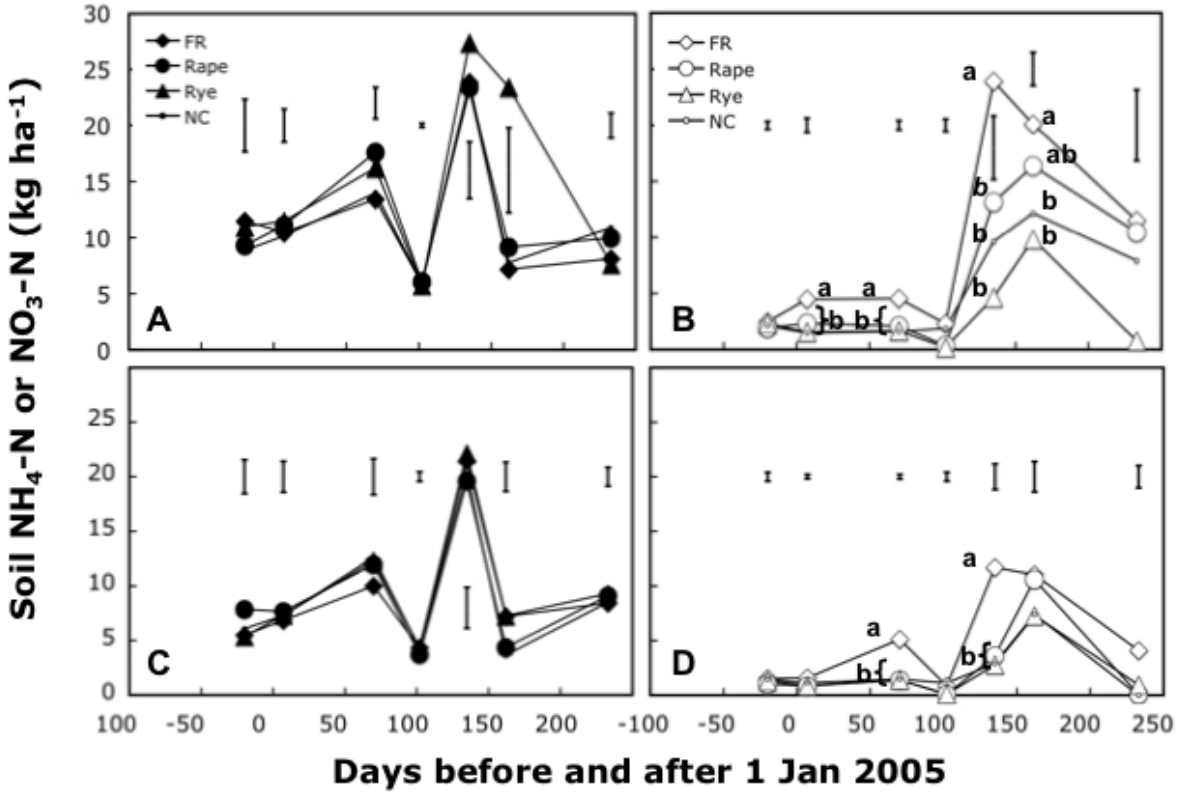

Fig. 3.8. Expt. 2: Ammonium (filled symbols) and nitrate (unfilled symbols) at 0-15 cm (A,B) and 15-30 cm (C,D) depths at CMREC. Vertical bars are average SEM. Mean values with the same letter are not statistically different at ($P<0.05$; italic, $P<0.10$), using Tukey-Kramer adjustment.

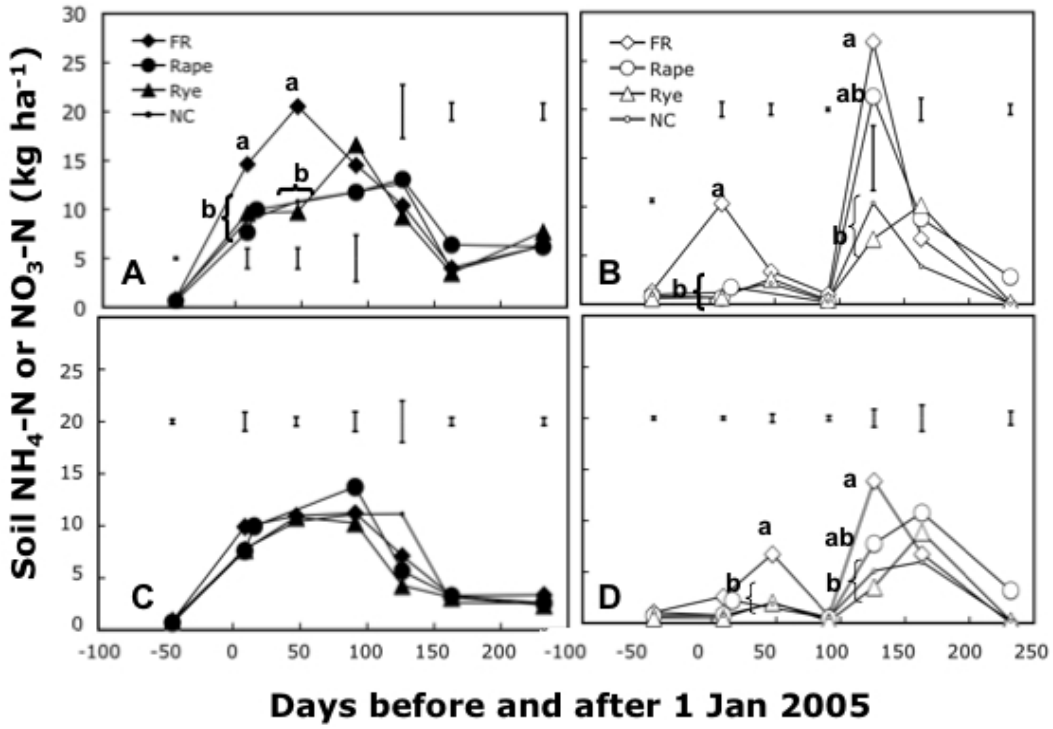

Fig. 3.9. Expt. 2: Ammonium (filled symbols) and nitrate (unfilled symbols) at 0-15 cm (A,B) and 15-30 cm (C,D) depths at LESREC. Vertical bars are average SEM. Mean values with the same letter are not statistically different at ($P<0.05$; italic, $P<0.10$), using Tukey-Kramer adjustment.

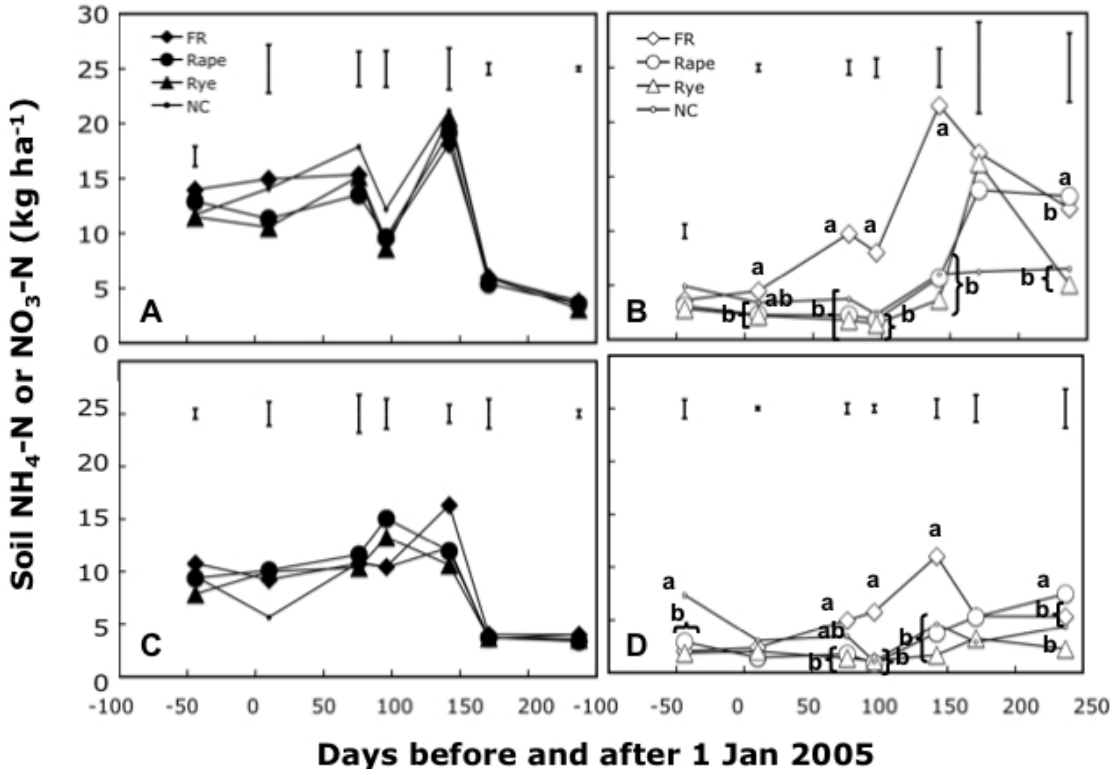

Fig. 3.10. Expt. 2: Ammonium (filled symbols) and nitrate (unfilled symbols) at 0-15 cm (A,B) and 15-30 cm (C,D) depths at WREC. Vertical bars are average SEM. Mean values with the same letter are not statistically different at ($P<0.05$; italic, $P<0.10$), using Tukey-Kramer adjustment.

In late winter to early spring (January to March) soil NO_3-N concentration in the (winter-killed) forage radish treatment plots at all sites significantly exceeded that in the control and (over-wintering) rape and rye treatments plots at both soil depths, with significant differences appearing earliest in the 0-15 cm layer (Figs. 3.8-3.10). During this period, surface (0-15 cm) soil NO_3-N concentrations in the forage radish plots exceeded those at the 15-30 cm layer at all sites, with no differences by soil depth for the other treatments. This trend continued at WREC (silt loam textural class) in April, but in the loamy sands at CMREC, NO_3-N in both soil layers in April was reduced to nearly zero, when sampling followed heavy rains (Fig. 3.2). Forage radish plot soil NO_3-N concentrations peaked at all sites in May, at around 25 and 12 kg ha^{-1} in the 0-15 and 15-30 cm soil layers respectively, and were significantly greater than NO_3-N concentrations for other treatments (for both soil layers). The exception was at LESREC, where NO_3-N in the rape plots at both soil depths in May was not significantly different from that in the forage radish plots. In June, no cover crop treatment differences in soil NO_3-N concentration were observed in either soil layer at all sites, excepting in the 0-15 cm soil layer at CMREC, where NO_3-N in forage radish plots significantly exceeded that in the no cover and rye plots. In August, there were no treatment differences in either soil layer at all sites, except at WREC, where NO_3-N in the rape plots (in both soil layers) significantly exceeded that of the other treatments.

Fig. 3.11 summarizes inorganic nitrogen concentration in soils for Expt 2. Magnitude and timing of earliest N release was in the following order: forage radish > rape > rye. Nitrogen release from cover crop residues at different sites was in the

order: fine textured soil with no-till management <coarse-textured soils with no till < coarse-textured soils with conventional tillage. Analysis of total soluble organic N measured in soils sampled in June of Expt. 2 did not identify any treatment differences (Fig. 3.12).

Early main crop N response

In Expt. 1 at CMREC, young soybean plants (harvested in late May, 14 days after planting prior to nodule formation) were significantly ($p<0.05$ adj=Tukey) larger and took up significantly more nitrogen in forage radish plots (Table 3.6). At WREC, no treatment effects of cover crop were observed in the growth of immature soybean plants (sampled in early June, 12 days after planting).

In Expt. 2, at CMREC, V6 stage corn plants had significantly greater dry weight and took up more N in the forage radish plots and rape plots than in the rye and no cover plots. At LESREC, V6 stage corn plants had significantly more dry matter and took up significantly more N in the rape plots compared to those in the no cover and rye plots. There was no statistical difference in the dry matter of V6 corn plants at WREC, but plant N uptake by corn in rye plots significantly exceeded that in the forage radish and no cover plots. Because corn plots received side-dress applications of fertilizer soon after sampling V6 stage plants, any influence of early N availability on corn growth was masked by the time of grain harvest, when no yield differences due to treatment were observed.

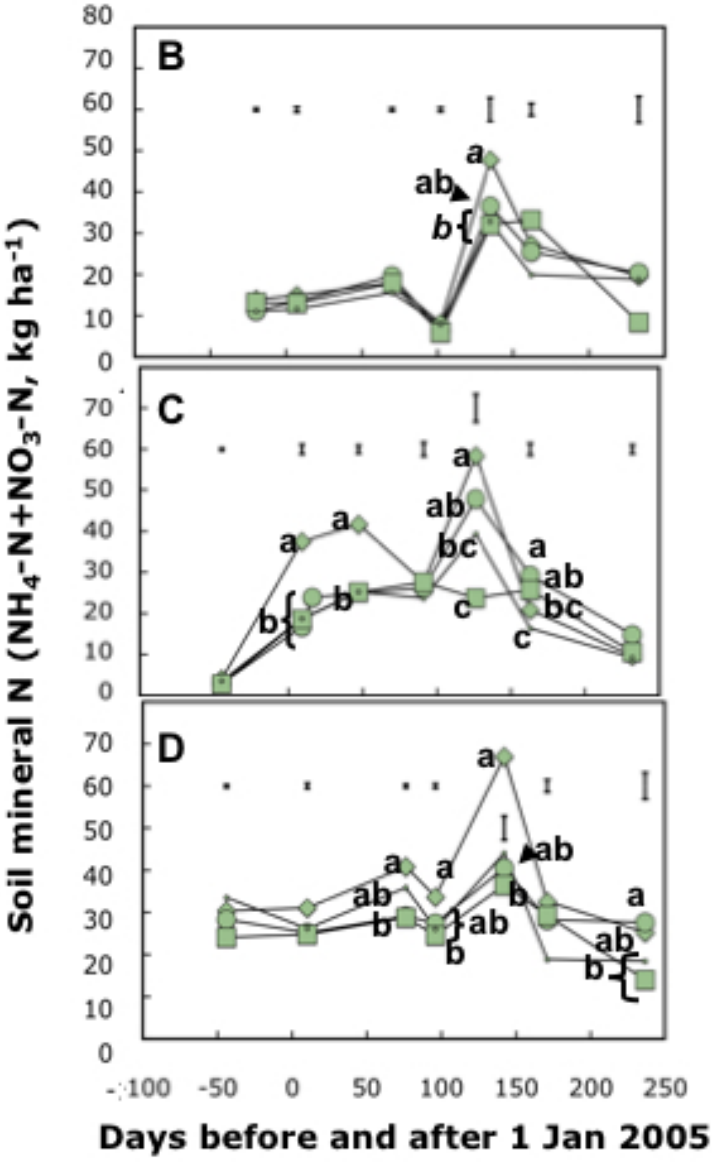

Fig. 3.11. Expt. 2: Soil mineral nitrogen (0-30 cm) at CMREC (B), LESREC (C) and WREC (D). Symbols are the same for each location as in previous figures. Vertical bars are average SEM. Mean values with the same letter are not statistically different at ($P<0.05$), using Tukey-Kramer adjustment.

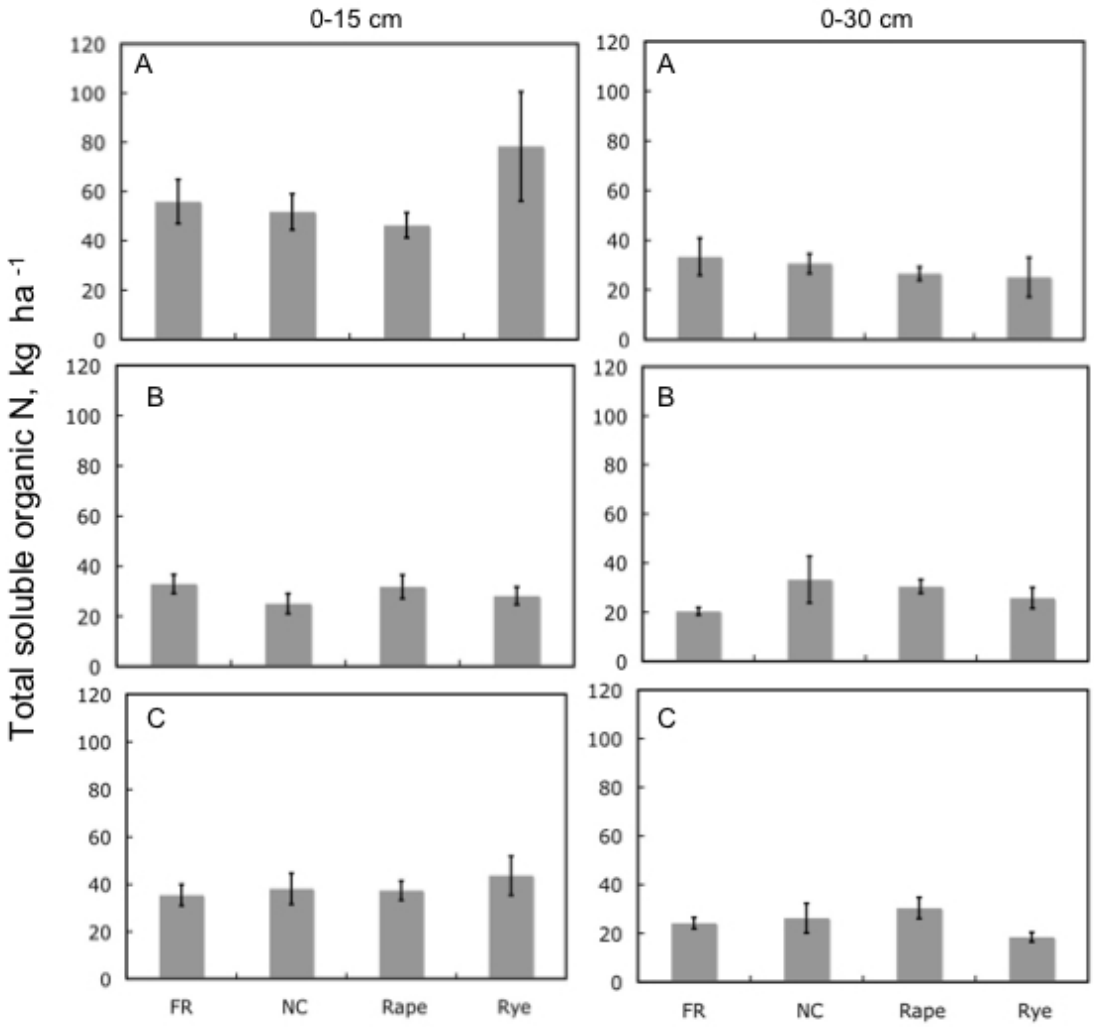

Fig. 3.12. Total soluble organic N measured in soils sampled in mid-June 2005 at CMREC (A), LESREC, (B), and WREC (C). Vertical bars are SEM (n=4).

Table 3.6. Dry matter and N content of 2-week old soybean seedlings (Expt. 1) and V6 corn shoots (Expt. 2).

Location	Cover crop plot	Biomass dwt	N uptake
Expt. 1: immature soybeans[†]			
		g plant^{-1}	mg plant^{-1}
CMREC	Forage Radish	0.25 (0.009)[‡] a[§]	14 (0.9) a
	No Cover	0.22 (0.006) b	11 (0.6) b
WREC	Forage Radish	0.20 (0.003) a	¶
	Rye	0.18 (0.014) a	¶
	No Cover	0.18 (0.012) a	¶
Expt. 2: immature corn[#]			
		g plant^{-1}	mg plant^{-1}
CMREC	Forage Radish	3.6 (0.2) a	98 (8) a
	Rape	¶	¶
	Rye	1.0 (0.1) c	30 (2) c
	No Cover	2.6 (0.4) b	63 (11) b
LESREC	Forage Radish	2.8 (0.4) ab	55 (11) ab
	Rape	3.7 (0.7) a	85 (27) a
	Rye	2.0 (0.3) b	44 (6) b
	No Cover	2.3 (0.4) b	45 (8) b
WREC	Forage Radish	2.7 (0.2) a	54 (10) bc
	Rape	2.8 (0.4) a	75 (15) ab
	Rye	2.6 (0.5) a	77 (24) a
	No Cover	2.1 (0.3) a	30 (5) c

[†]Average per plant values (sampled from 2 m length of row, ~16 per block) are presented.

[‡] Numbers in parenthesis are SEM, n=4

[§] Within location, numbers followed by different letters are statistically different ($p<0.05$), adjusted using Tukey-Kramer.

¶ Not determined.

[#] Average per plant values (16 plants per block were randomly sampled) are presented.

Discussion

Dry matter production and N uptake by Brassica and rye cover crops in this study were consistent with that observed by others (Vos and van der Putten, 1997; Thorup-Kristensen, 1994; Thorup-Kristensen et al., 2003) (Figs. 3.4 and 3.5). In addition to sowing date, factors such as adequate moisture and ensuring sufficient N availability are also important for fall growth by Brassicas (Vos and van der Putten (1997), Jensen et al. (1997); Hocking et al. (1997); Trintrousot, 2000a). In Expt. 2, N uptake and biomass production at WREC (planted September 24 and harvested after only 7 weeks) was comparable to that at CMREC and LESREC (planted a month earlier and harvested after 9 and 11 weeks, respectively). The rapid growth at WREC relative to the other sites might be due to greater residual N in the soil profile and higher moisture content in the fine textured soils at WREC compared to the well-drained loamy sands at CMREC and LESREC, particularly during October when rainfall was less than the monthly long-term normal.

Several factors might explain the differences observed in fall-to-spring N uptake and N retention by cover crops in Expt. 1 and Expt 2. Nitrogen loss from rape and rye tissues in Expt. 1 might be explained by leaf drop and N leaching as a result of higher than average precipitation and colder than average temperatures in the winter/early spring. Cover crop harvest date may have also influenced our results, since the N concentration of the covers crops studied are expected to decrease (due to slowing N uptake) during late growth stages (Vos and van der Putten, 1997; Malagoli, et al., 2005; Hocking et al.,1997). In Expt. 1, cover crop spring biomass harvest took place when rye was in boot stage and when rape plants had fully

flowered and were forming immature seedpods. By comparison, the cover crops in Expt. 2 were slightly less mature. When killed in spring, rye was not quite at boot stage, and rape had just begun to flower. Planting cover crops later (by 2 weeks or more) in Expt. 2 compared to Expt 1. may have improved the winter hardiness (and therefore N retention) of over-wintering covers, which would be similar to observations made by Vos and van der Putten (1997).

Even though our sampling methods were not suited for making estimations regarding leaching loss, in Expt. 2., the effect of intense precipitation events on soil inorganic N concentration on certain dates in our study was obvious, especially at sites with coarse textured soils. For example, in soil NO_3-N at CMREC was reduced to nearly zero mg kg^{-1} from 0 to 30 cm when the April sampling date followed two weeks of precipitation totaling > 40 mm, with most rainfall occurring in a single event 12 days prior to sampling. Similarly, at LESREC, > 60 mm of rain fell, mostly as one >50 mm rain event in the week prior to the April sampling date, which appeared to have the effect of reducing soil NO_3-N in both soil layers. At CMREC, NH_4-N also appeared to be susceptible to leaching losses. In Expt. 2, heavy rains seem to be the most plausible explanation for the sharp decrease in NH_4-N concentration observed on the April sampling date. Rainfall influences on NH_4-N concentrations were not observed at LESREC or WREC.

Several factors might explain why cover crop treatment differences were observed in soil mineral N concentration in spring of Expt. 2 but not Expt. 1:

First, it is possible that infrequent sampling (as well as the timing of sampling close to rainfall events) could have caused us to miss peak periods of mineralization

during Expt. 1 that were more likely to be captured by increasing the number of sampling dates and sampling over longer period of time (from late winter through mid-June) in Expt. 2.

Second, based on soil N status (i.e, whether or not soil N levels are limiting to soil microbial function) nitrogen from decomposing cover crop residues in spring may become immobilized one year while mineralizing in the next, or vice versa, even when C/N ratios of decomposing plant residues are low (Thorup-Kristensen, 1993; Bengtsson et al., 2003; Justes et al., 1999). We suspect that based on previous cropping history—(BARC, potatoes and fallow; CMREC and LESREC, wheat; and WREC, corn)—that N from decomposing residues became immobilized by the soil microbial biomass in Expt. 1. By comparison, cover crops in Expt. 2 were planted into a relatively N-enriched environment—either a mowed soybean crop (CMREC, WREC) or into an area lacking carbonaceous residues that received an application of 120 kg ha^{-1} N fertilizer (LESREC)—which would have increased the likelihood of more immediate and extensive N mineralization from cover crops the following spring.

Last, if soils were often saturated as they began to warm in spring, denitrification rates could have intensified, especially during the initial decomposition of non-homogeneously distributed, low C/N ratio (N-rich) cover crop residues (Aulakh et al., 1991; Shelton et al., 2000; Magid et al., 2006). It is possible that N might have been lost from surface soils during Expt. 1 compared to Expt. 2 because conditions in the former were more favorable for denitrification; in May of Expt. 1, soil temperatures averaged >20 °C and there were multiple days of heavy rainfall

during the month. By comparison, May temperatures in Expt. 2 were normal or slightly cooler than average (~15 °C), with fewer days of rainfall (CMREC, 13; LESREC, 8; WREC, 8), which allows us to hypothesize that that cover crop decomposition was not quite as rapid and that less N was lost from residues via denitrification in the second year. In accounting for lower soil nitrate-N concentrations from spring-killed rape and rye residues at WREC relative to the other field sites, it could be argued denitrification might have been encouraged by the combination of no-till management and increased persistence of moist conditions in WREC's fine textured soils, similar to observations made by Doran (1980).

Compared to no-till management, aerobic activity is stimulated to a deeper soil depth with conventional tillage (Doran, 1980); this may be reflected by our May results for Expt, 2, when nitrification in the 15-30 cm layer for most treatments (excepting forage radish) was greater at LESREC than at CMREC and WREC. Elevated mineral N observed at in August of Expt. 2 at WREC and at CMREC (from rape and forage radish treatments especially) suggests that mineralization from cover crop materials at sites managed using no-till was still ongoing. Overall, nitrogen turnover seemed to be slowest at WREC. This may have been partly due to temporary protection of N in residues from microbial attack, via complex formation between residues and fine textured particles as described by Bending et al. (1998),

In both years, cover crop N had a positive influence on early season growth of subsequent main crops at CMREC (soybeans and corn), LESREC (corn) and WREC (corn). Low-density stands of corn in the rye plots at WREC in Expt. 2 resulted greater dry matter in individual plants (but not necessarily more dry matter per unit

land area) separate from cover crop influence. Overall, earlier release of N from forage radish and rape residues and response by the young crops suggests that that the relative potential for early N availability to main crops is greater from forage radish and rape than from rye. The only instance where evidence (elevated soil NH_4^+-N concentrations) existed that glucosinolate hydrolysis from Brassicas might be causing nitrification inhibition was in the forage radish plots (January and February) at LESREC.

Frost-killed tissues of forage radish appeared to provide the earliest appreciable, significant quantities of mineralized N (as early as January). Our results suggest that nitrogen release from forage radish may peak in mid-May. Corn, normally planted in early to mid April, may benefit from this early N release. The period of corn's greatest N need may come after the peak of N release from the forage radish residues. Early release of N from forage radish highlights advantages of planting cover crop mixes of winter-killed and over-wintering species or using early-planted main crops to achieve best N use efficiency by main crops.

Conclusion

Fall N uptake by forage radish and rape in this study was comparable to or greater than that of rye. Sampling soils monthly starting in late fall-early winter and continuing into spring allowed us to observe and better characterize peaks in N turnover from the different study sites in Expt. 2 compared to Expt. 1, when samples were taken only from March-June. Decomposition of cover crop residues led to N immobilization in some years and N mineralization in others, which was probably

related to soil N status when cover crops were growing, C and N status when their residues were decomposing, and the maturity of cover crops when killed in spring. In two coarse textured soils, intense rainfall appeared to be capable of leaching most the mineral N below the depth of our soil sampling. Our data suggest that N in frozen, decomposing forage radish residues in spring is likely to be at risk for leaching in coarse-textured soils. Since our results (in Expt. 1) on using the forage radish and rye mixture as a way to conserve N mineralized from the radish tissue in late winter/early were inconclusive, additional investigation into this mixture is warranted. Even with the potential risk for inorganic N leaching following the winter-kill of forage radish, this cover crop consistently provided the greatest N availability to main crops in spring, indicating that a substantial portion of N from forage radish residues can be retained in surface soils until it can be used by subsequent main crops. In summary, our main finding that greatest possible N release in spring comes from forage radish, followed by rape and then rye, is consistent with research conducted elsewhere (Thorup-Kristensen, 1993; Vos and van der Putten, 1997). Peak nitrogen release from forage radish residues in May could coincide with the acceleration of corn N uptake beginning about 1 month after planting in early April. Important questions not addressed by this research include the possible influence of glucosinolate degradation in decomposing rape and forage radish residues on nitrification inhibition as well as the effect of high calcium content in Brassica cover crops on nitrification and denitrification.

Chapter 4: Mineralization of C and N from forage radish, rape, and rye cover crop residues incubated in fine-and coarse-textured soils.

Abstract

A 48-day incubation study was set up to compare C and N mineralization from shoot and root residues of forage radish (*Raphanus sativus*, L.) and rape (*Brassica napus,* L.), since most investigations of N turnover from these plants have only used plant shoots. Contrasting soils—silt loam and loamy sand—were used to compare differences in C and N mineralization based on soil types when amended with different residues. Evolution of C was rapid in both soils within 24 h following incorporation of plant materials into soil but declined thereafter, and the C evolution rate was low from day 16 to the end of the study. A two-pool equation (exponential for the labile pool + linear for the recalcitrant pool) was used to fit curves to accumulative C mineralization data as well as identify differences in pool size values and rates of pool decomposition. Average cumulative C mineralization from all materials was approximately 450 g kg^{-1} added residue C over the 48-day period. Modest but statistically significant net N mineralization was evident in the silt loam amended with forage radish shoot (C/N ratio of 12) and in the Cedartown loamy sand amended with rape shoot (C/N ratio of 16) beginning at 4 days following incorporation. For the other residues (C/N ratios of $\geq$ 21), including rye shoot, no significant net mineral N increase was observed in comparison to the non-amended control soil. As much as 29 to 37% of N added to soil with the forage radish and rape shoot residues was measured as soil mineral N on the last day of the study.

Introduction

Rapid and substantial nitrogen (N) turnover from decomposing winter cover crops that is timed with the period of greatest N uptake by subsequent main crops in spring will promote economically and environmentally sound nutrient management. In the state of Maryland (USA), a government cost-share assistance program encourages the planting of winter cover crops to reduce nitrate leaching from farmland draining into the Chesapeake Bay watershed (Maryland Agricultural Water Quality Cost-Share Program, 2006). Winter cover crops in the Brassicacea family have recently been included in the program. Among the Brassicas, forage radish (*Raphanus sativus* L.) and rape (*Brassica napus* L.), have been reported to take up as much as 250 kg N ha^{-1} if planted by late August to mid-September (Thorup Kristensen, 1994, Vos and van der Putten, 2001; Eichler et al., 2004; Chapter 3, this thesis). Once these cover crops are killed, a large amount of nitrogen is returned in organic form to the soil. Farmers would benefit from knowing how quickly and how much N will become available during the growing season. In the mid-Atlantic region, the standard cover crop grown in the region—rye (*Secale cereale* L.)—if not killed by the time it reaches reproductive stages of growth, can immobilize rather than release N from its residues early in the main crop growing season (Clark et al., 1994). Little research comparing mineralization trends from forage radish, rape, and rye in the mid-Atlantic region has been conducted. In a field study in Denmark, Thorup-Kristensen (1994) reported that the N mineralized in time for uptake by a spring-planted barley crop was ~50% of total fall nitrogen uptake for forage radish, which

was killed by freezing temperatures in winter, and ~30% of N in rape and rye plants, which were killed in spring by rotovating.

Nitrogen mineralization from cover crop materials in the field is affected by several factors including: differences in soil temperature, soil moisture, soil aeration, soil texture and soil microbial biomass; residue biochemical quality; residue particle size and residue-soil contact; and drying, wetting and freezing effects on soil and plant material (Cabrera et al., 2005). Extensive research into biochemical quality of plant materials has concluded that, assuming the phenol and lignin concentrations are relatively low, N concentration or C/N ratios of plant materials provide enough information to be able to predict net effects of most cover crop residues on soil mineral N dynamics (Trintrousot et al., 2000a). Laboratory incubations of plant materials are often used to compare mineralization rates of different plant materials under controlled conditions. However, few lab studies have used forage radish residues and few have used rape plants of similar maturity to what would be found in our region just prior to their being killed in spring. In addition, most incubation studies have used only above-ground portions of plants, even though root N contributions can be substantial and shoot and root tissues can differ substantially in terms of their N turnover (Malpassi et al, 2000; Bending and Turner, 1999.) This paper presents the results of a 48-day incubation experiment that compared C and N mineralization from roots and shoots of forage radish and rape and shoots of rye, using two contrasting soils (a silt loam and a loamy sand).

Materials and methods

Soils

Bulk soil samples were collected (using a spade) on 05 November 2005 from the Ap horizon at two experimental sites in Beltsville, Maryland (USA). Elkton silt loam (fine-silty, mixed, active, mesic Typic Endoaquult) was collected from USDA's Beltsville Agricultural Research Center (BARC). This soil contained 220 mg g^{-1} OM, 240 mg g^{-1} clay, 490 mg kg^{-1} silt, and 270 mg g^{-1} of sand, and had a pH of 6.1. A Cedartown loamy sand (siliceous, mesic psammentic Hapludult) was collected from the University of Maryland's Central Maryland Research and Education Center (CMREC). This soil contained 130 mg g^{-1} organic matter, 600 mg g^{-1} clay, 160 mg g^{-1} silt and 780 mg g^{-1} sand and had a pH of 5.6. Both soils were collected after corn harvest from fields in a corn-soybean rotation from an area on which no fall cover crop had been planted. Each soil was sieved (8 mm) in a field-moist state, and refrigerated at 4 °C in sealed doubled plastic bags before beginning the incubation study within 7 days from the date of collection. Six sub-samples were used to determine gravimetric soil water content. Field moist soil was used for the incubation.

Plant materials

Plant samples (root or shoot tissues) were harvested from several field experiments with similar growing conditions. Forage radish (var. 'Dichon') was sampled in fall (October-November) 2004 prior to being killed by freezing conditions. Rape (var. 'Dwarf Essex') and rye (var. 'Wheeler') were sampled in April 2005 prior to being killed using chemical and/or mechanical methods. The following plant samples were

used in this study: roots and shoots of forage radish (the part of forage radish's fleshy taproot that grows aboveground was considered to be part of the root material); roots and shoots of rape; and rye shoots. All plant samples were dried in a forced-draft oven (60 °C), milled, sieved (< 1 mm) and stored in polyethylene vials at room temperature until being used in this study.

Incubation set-up

To approximate the plant loading rates of cover crop residues in the upper 1 cm of soil in a no-till system, plant material was added to soil at a rate of 5 g of plant dry matter per 100 g dry weight equivalent of soil. Each plant tissue-soil mixture was transferred to a separate clean plastic bag. Based on preliminary determination of the saturated water content of each soil and type of tissues, deionized-distilled water was added to bring the plant tissue-soil mixture in the bag to 60% water-filled pore space, a water content considered optimal for aerobic decomposition (WFPS) (Linn and Doran, 1984). Bags were sealed and lightly kneaded until the contents appeared to be uniformly mixed and moistened. The control soil (soil not amended with plant material) was subjected to similar manipulation.

Each soil-residue mixture was weighed into eight 20 ml plastic beakers (10.0 g dry weight equivalent, each) and initial weights recorded. Each beaker was tapped gently 3 times to settle the contents. One soil-filled beaker per treatment was placed immediately into a forced-draft oven (60 °C) to halt mineralization. The remaining 7 beakers were placed in the same 1.6 L chamber with a sealing lid. A 20 ml plastic beaker containing 10 ml of deionized-distilled water was placed in each chamber to

maintain high relative humidity and prevent soils from drying out. To determine rates of microbial respiration, a 20 ml beaker containing 10 ml of 2 *M* NaOH as an alkali trap was also included in each chamber. Two "blank" chambers containing vials of NaOH and deionized-distilled water but no soil were also incubated to determine background CO_2 levels. This method was replicated 4 times for each plant amended soil treatment, and 8 times for control soils and no-soil chambers. The chambers holding beakers of soil, water, and NaOH were incubated in the dark at 25.0 ± 0.2 °C. After 1, 2, 4, 8, 16, 32, and 48 days, one beaker containing soil was removed from each chamber for analysis and was weighed moist before being dried at 60 °C. Based on gravimetric determination of moisture lost upon drying, deionized-distilled water (0.5 ml) was added drop-wise to each remaining beaker in the sealed chambers at days 12 and 24 to maintain moisture levels. All chambers were opened for 30 seconds at least every 4 days to replenish oxygen levels. Dried soils were ground, sieved (< 2 mm) and stored in polyethylene plastic vials at room temperature. The alkali trap in each chamber was replaced each time a beaker of soil was removed.

Analytic determinations

Total C and N contents of plant materials and soils prior to incubation were determined using high temperature combustion analysis (CHN 2000- LECO Corporation, St. Joseph, Michigan; Campbell, 1992). Soil nutrient content was determined using Mehlich-3 extraction (Mehlich, 1984). To determine C respiration rate, NaOH from the alkali trap was transferred quantitatively to an Erlenmeyer flask

and carbonates in the trap were precipitated using 1 *M* $BaCl_2$. Remaining OH^- was determined by back titration with 1 *M* HCl, using a manually operated micro-burette and phenolphtlalein as indicator.

For soil mineral N analysis, three grams of dried, ground, sieved soil was shaken with 20 ml of 0.5 *M* K_2SO_4 for 30 minutes at 100 rpm, centrifuged for 2 minutes at 3000 rpm, and then filtered (Q2 filter papers, Fisher Sci., International, Hampton, NH). Filtrates were refrigerated and analyzed within 24 hours from extraction. Ammonium concentration was determined using an Orion 9512 ammonia specific gas-sensitive electrode (Banwart et al., 1972). Standards (ranging in concentration from 0 to100 mg N L^{-1}) were prepared by diluting NH_4Cl in 0.5 *M* K_2SO_4. One ml of 5 *M* NaOH ionic strength adjusting solution with thymolpthalein pH indicator (blue at pH 13) was added to 10.0 ml of sample to raise sample pH above 13. Millivolt readings were recorded for stirred samples and standards when the change in mV slowed to <1mV s^{-1}.

Nitrate concentration was determined in filtered samples by cadmium reduction using a Technicon Autoanalyzer II flow injection analyzer (Technicon Industrial Method No. 487-77A, 1977). Denitrification was not measured, because it was expected to be minimal at 60% WFPS (Linn and Doran, 1984; Aulakh et al., 1991).

The optical density of soil extracts was measured at 410 nm by spectrophotometer as an indication of soluble humic compounds (Islam and Weil, 1998).

Calculation of results

Respiration rate (g CO_2 evolved kg residue C^{-1} day^{-1}) was calculated by subtracting the background CO_2 (from traps in the "blank" and in the control soil containers) from CO_2 evolved in residue-amended soil, adjusting values to account for days elapsed since the trap was added to the incubation, the number of remaining beakers containing soil within incubation containers during that period, and the amount of carbon added with each residue. The kinetics of cumulative C mineralization were estimated using a two-pool (exponential plus linear) equation:

$$CO_2\text{-}C = C_f (1-e^{-kf*t}) + C_s k_s t \qquad (1)$$

where t is days; C_f represents a labile substrate pool that has a first order rate of decomposition described by kf [in $days^{-1}$]; and $C_s k_s$ represents a linear rate of mineralization from a "slow pool" of more resistant residues. Though the slow pool and its associated rate are conceptually separate in physical terms, in a zero-order model they are not mathematically independent, therefore, we always present $C_s k_s$ together as a single number (Wang et al., 2004).

Net mineralization or immobilization was calculated by taking the difference of mineral N in plant-amended soil treatments and that in the control soil determined for each sample removal date. These values were then expressed in terms of mg N per kg of added N in each residue or g N per kg C added in each residue to allow direct comparison between treatments (Trintrousot et al., 2000a). Our use of the

term “net mineralization” should not be confused with its use by others who are referring to a calculation of the difference of mineral N at time zero and that at the time of sampling. In this paper, the term “final-initial” corresponds to a calculation of the difference of mineral N at day 48 minus that at day 0. Even though our experimental design does not allow for a true estimation of mineral N released cumulatively over time, a figure presenting “additive” N (in mg kg soil^{-1})—e.g., soil mineral N on day 1 plus soil mineral N on day 2 plus soil mineral N on day 4, etc.—is used to compare N turnover from the different residues.

Statistical calculations

C and N mineralization treatment means were compared using the MIXED procedure in SAS (Version 9.1, SAS Institute, Cary NC, 2005). The model statement tested for soil*treatment interactions for the CO_2 curve parameters, and for three-way soil type*treatment*day interactions for the mineral N data. Differences were considered significant at $P \leq 0.05$, using the Tukey-Kramer adjustment. Curve-fitting parameters describing cumulative CO_2 evolution were generated using a method that minimized the residual sum of squares, as generated by the Solver tool in Excel (Microsoft, 2004). For a few cases, manual adjustment of the initial parameter values generated was needed to obtain non-negative pool size values for subsequent iterative runs of the data through the program, as described by Wang et al. (2004).

Results

Carbon and nitrogen composition of materials used in the incubation

Table 4.1 summarizes the carbon and nitrogen data obtained for the plant residues and soils used in the experiment. The C/N ratio for all residues used was ≤ 25. Brassica shoot residue C/N ratios were less than C/N ratios for their roots. The C/N ratio for rye shoots (24) was higher than the Brassica shoots and similar to that of Brassica roots. Addition of shoot dry matter contributed one-third to one-half more nitrogen than was added by incorporating root dry matter, with the exception of rye shoots, which contributed roughly the same amount of N to soil as adding Brassica roots.

Table 4.1. Characteristics of C and N in plants and soils.

Treatment	Plant Part	C/N Ratio	Total N mg g^{-1}	C added in materials used	N added in materials used	
				g C kg^{-1} residue C	g N kg^{-1} residue C	mg N kg^{-1} soil
Forage Radish	Shoot	12	31	371	84	1564
	Root	25	14	353	41	718
Rape	Shoot	16	26	408	63	1284
	Root	21	17	356	48	867
Rye	Shoot	24	17	416	43	858
Soil						
Elkton silt loam	--	10	1	--	--	1200
Cedartown loamy sand	--	12	1	--	--	1300

Visual and olfactory observations

Fungal mycelia (a fluffy white mold) quickly covered the surface of all the plant-amended soils (not the control soils) during the first two days of the incubation. Soils amended with Brassica tissues, particularly forage radish roots, gave off strong sulfurous odors that decreased somewhat during the incubation. Other soils gave off only an earthy odor.

Mineralized C and two-pool kinetic parameters

Figure 4.1 presents a comparison of cumulative C release as CO_2 from the plant-amended soil treatments, with values subtracted for CO_2 evolved from the control soil and from the "blank" containers. After rapid initial CO_2 release, respiration rates from all residues slowed between 8 and 16 days into the incubation.

A two-pool (first-order + zero order) equation was used to parameterize cumulative C mineralization kinetics, and gave very close fits for the measured data ($R^2 \geq 0.997$). There was a main effect of soil type on the labile pool size (C_f), which varied from 296 to 518 g kg^{-1} residue C. The only significant treatment effect observed for the C_f parameter was in the Cedartown loamy sand, where C_f for forage radish root exceeded that of rape shoot. There was a soil type*treatment interaction for the labile pool parameter k_f.

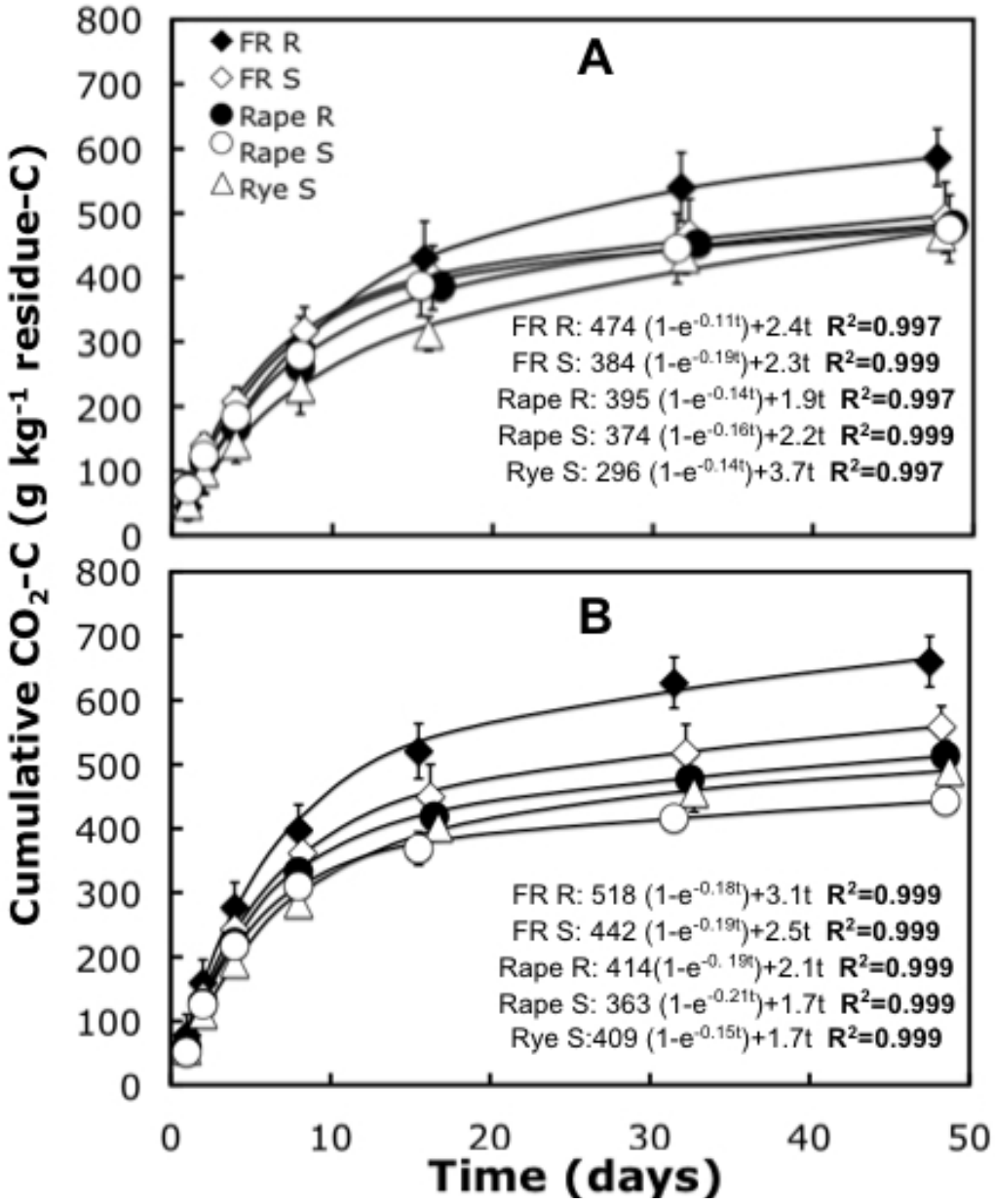

Fig. 4.1. Cumulative CO2 evolution (points=measured and lines=estimated) over time in (A) Elkton silt loam and (B) Cedartown loamy sand. Bars are SEM, n=4. Background CO2 and CO_2 evolved from control soil have been subtracted from data

Labile pool parameter (k_f) values ranged from 0.11 to 0.21 $days^{-1}$. The k_f value was significantly larger for rape shoot compared to that for rye shoot in the Cedartown loamy sand. For the slow pool parameter (C_s*k_s), there were no main effects of soil type or differences due to treatment type.

Mineralized N in contrasting soils

Generally, similar trends were observed for N mineralization for the different treatments but these patterns were not identical for the two contrasting soil types. Because of three-way soil type*day*treatment interactions details are presented separately by day for the two soil types.

Ammonium proportion

In both soils, the mineral N content (N_{min}=NH_4-N plus NO_3-N) of soil extracts was dominated by ammonium during the first four days of the incubation (Fig. 4.2). Later, the actual proportion of ammonium as mineral N (NH_4-N/N_{min}) differed somewhat among treatments and between the soils, but similar general trends were observed. In general, NH_4-N/N_{min} was initially about 50%, rose to near 90% or greater in the first 1 or 2 days, and then declined thereafter to <50%, except for soils amended with rye shoot and forage radish root, in which the decline of NH_4-N/Nmin began after day 16. The decline of NH_4-N/N_{min} was steepest for rape shoots in both soils; lowest levels (≤5%) of NH_4-N/N_{min} were observed for the rape shoot treatment at end of the incubation.

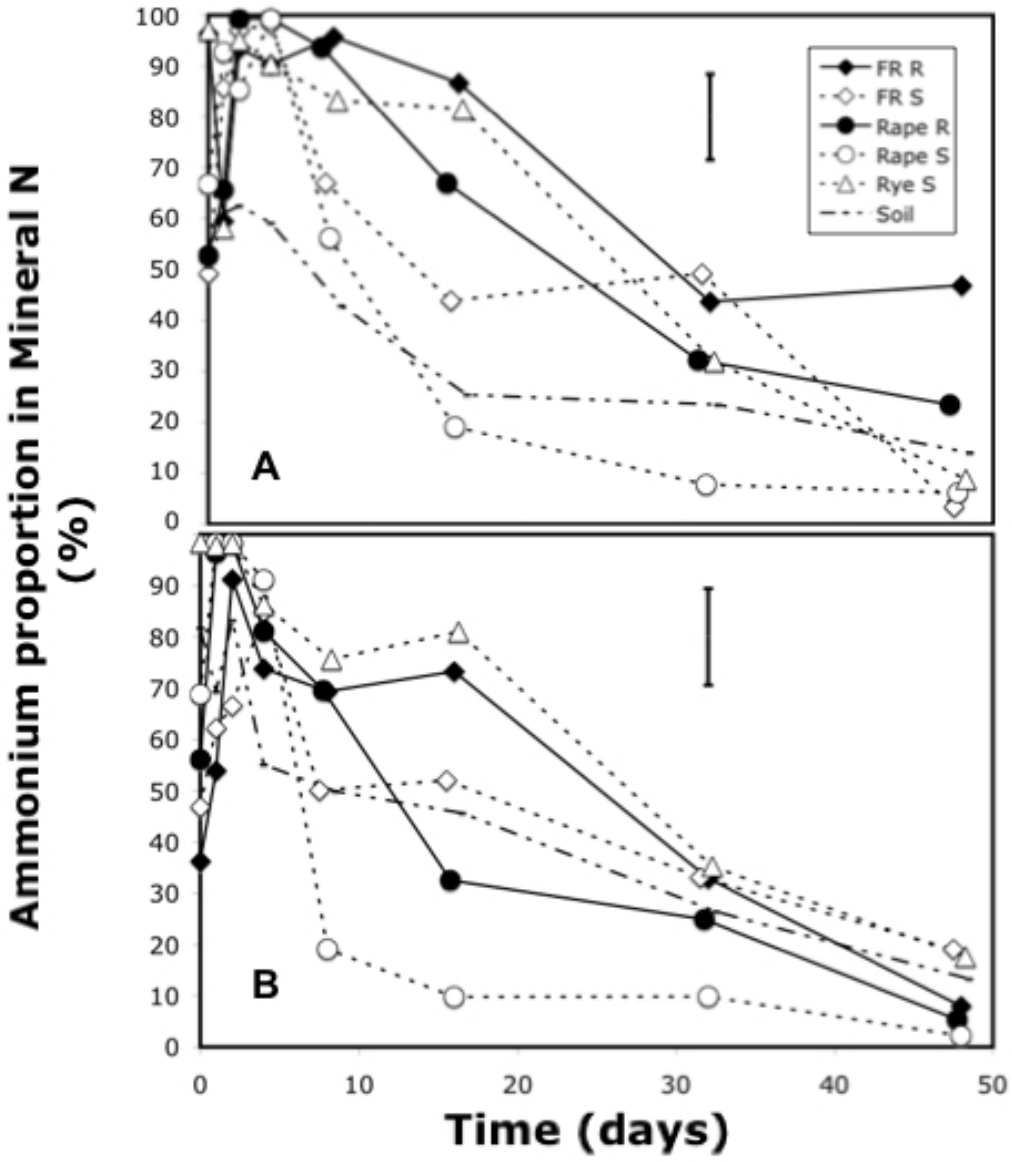

Fig. 4.2. Proportion of NH_4-N in mineral N extracted (expressed as %) from Elkton silt loam (A) and Cedartown sandy loam (B) control soil and plant-amended treatments. Data points are separated horizontally for clarity. Vertical bars indicate the mean SE (n=4; n=8 for control soils).

Soil N mineralization

The addition of residues to soils had varying effects on net mineralization throughout the incubation (Fig. 4.3). There was no significant net N mineralization or immobilization relative to the un-amended (control) soil for the first 4 days of the incubation. This initial period was followed by a period (which continued to the end of the study) of significant net N mineralization relative to the control soil for the forage radish shoot treatment in the Elkton silt loam, and for the rape shoot treatment in the Cedartown loamy sand. For the other treatments, there was no statistical difference of mineralization or immobilization of N compared to each other or the control soil during the incubation. Of the N added to soils with plant residues, the maximum amount of mineral N observed in soil at the end of the incubation was close to 30% (forage radish shoot treatment in the Elkton silt loam) or 40% (rape shoot treatment in the Cedartown loamy sand) (Table 4.2). Additive mineral N was generally greatest from the lowest C/N ratio materials (shoots of forage radish and rape) (Fig. 4.4).

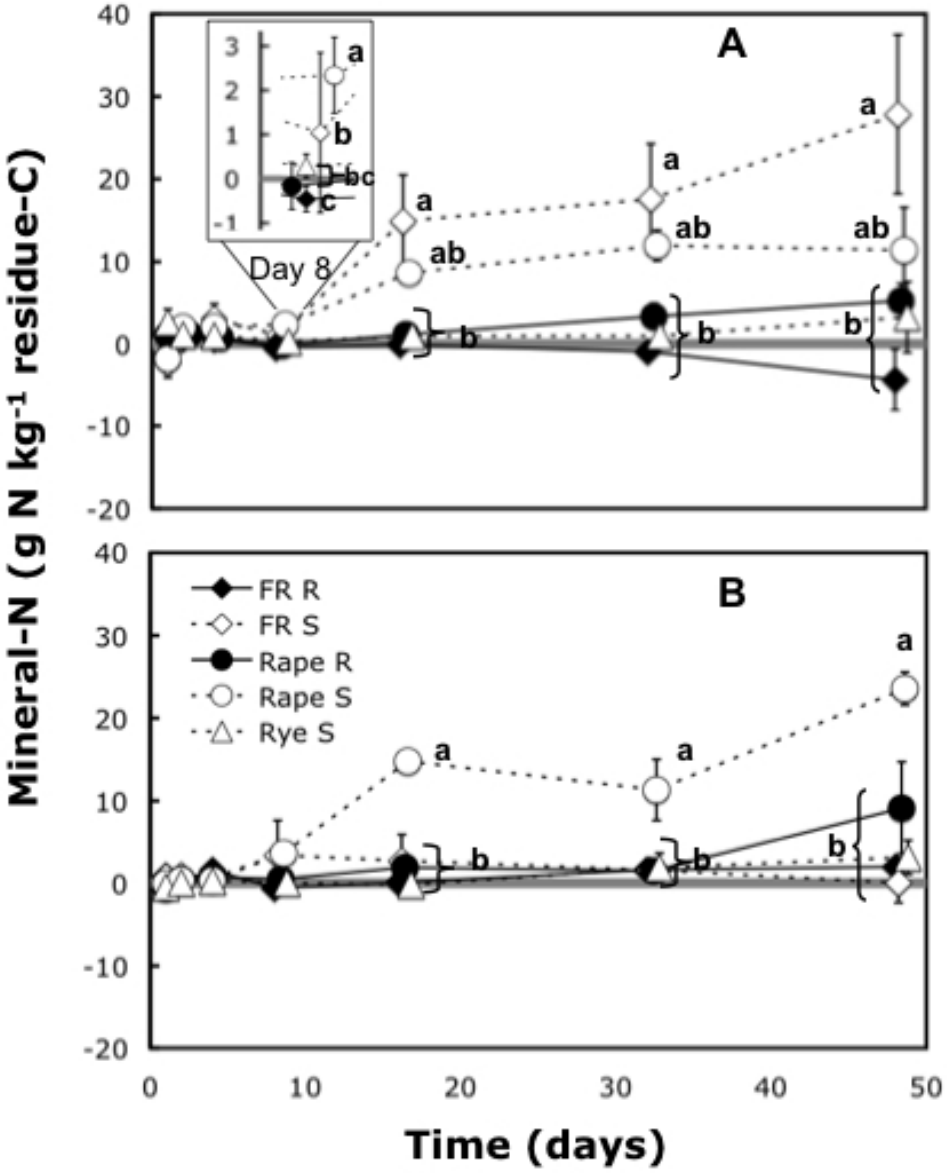

Fig. 4.3. Mineral N (NH_4-N + NO_3-N) in amended Elkton silt loam (A) and Cedartown loamy sand (B). Immobilization relative to control soil values is expressed as negative values, below the gray line. Data points are separated horizontally for clarity. Error bars are SEM, n=4; n=8 for control soil. Within each day, different letters next to mean values indicate statistical differences ($P \leq 0.05$, using Tukey-Kramer adjustment).

Table 4.2. Milligrams of N mineralized per g of N added with residue† on selected days.

Treatment	day 8	day 16	day 32	day 48
Elkton Silt loam				
FR‡ root	-14.6	-1.8	-31.2	-106.5
FR shoot	16.5	176.3	208.2	293.5
Rape root	-3.3	23.3	68.6	142.7
Rape shoot	37.0	136.8	189.8	105.2
Rye shoot	7.0	23.2	22.6	78.1
Cedartown loamy sand				
FR root	-14.1	2.32	39.7	48.7
FR shoot	40.0	32.3	104.8	28.6
Rape root	10.1	39.3	30.7	187.6
Rape shoot	57.8	234.5	179.4	374.0
Rye shoot	1.0	-4.6	60.5	75.5

† Values for nitrogen mineralized from un-amended (control) soil have been subtracted from the data presented.

‡ "FR" is an abbreviation for forage radish.

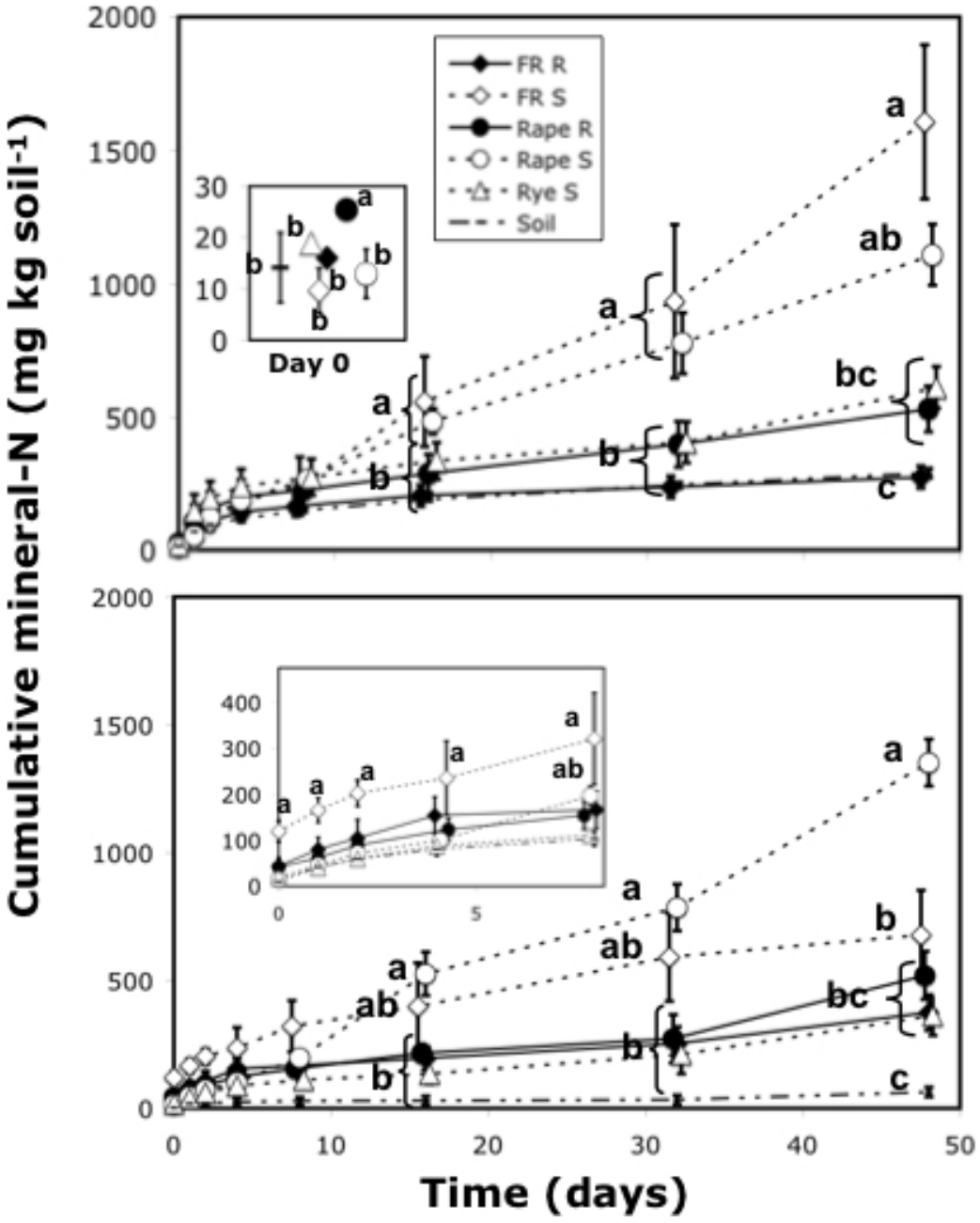

Fig. 4.4. Additive mineral N (NH_4-N + NO_3-N) in amended Elkton silt loam (above) and Cedartown loamy sand (below). Data points are separated horizontally for clarity. Error bars are SEM, n=4; n=8 for control soil. Within each day, means followed by the different letters are statistically different ($P \leq 0.05$, using Tukey-Kramer adjustment.

Compared to initial soil mineral N concentrations in soil on day 0, final mineral N concentration (on day 48) was significantly greater than that in the control soils for the forage radish shoot (Elkton silt loam) and rape shoot (Cedartown loamy sand) treatments (Table 4.3).

Optical density of extracts

Throughout the incubation, the forage radish shoot and root treatment extracts had a significantly darker yellow-brown color (as compared by measuring absorbance values at 410 nm) compared to the other treatments for both soils (Fig. 4.5). Solutions from soils amended with rape and rye residues were colored to a lesser degree. Un-amended soil extracts remained nearly colorless throughout the incubation for both soils. In general, the intensity of the coloration of forage radish root and shoot treatment soil extracts was significantly higher in the Cedartown loamy sand compared to the Elkton silt loam. The intensity of plant amended extract colors declined throughout the incubation for both soils. By the end of the incubation, there was no difference statistically in the color of the extracts from all treatments for the Elkton silt loam, while forage radish root and shoot extracts were still significantly more colored than the other treatments in the Cedartown loamy sand.

Table 4.3. Calculation of final (day 48)-initial (day 0) mineral N

Treatment	Plant Part	Final-initial
		mg N kg^{-1} soil
	Elkton silt loam	
Forage radish	Shoot	661 (229)[†] a[‡]
	Root	21 (9) b
Rape	Shoot	318 (102) b
	Root	107 (49) b
Rye	Shoot	187 (37) b
Soil	--	27 (8) b
	Cedartown loamy sand	
Forage radish	Shoot	4 (28) b
	Root	76 (53) b
Rape	Shoot	551 (40) a
	Root	206 (102) b
Rye	Shoot	132 (69) b
Soil	--	74 (30) b

[†] Numbers in parentheses are SEM, n=4; n=8 for control soil.

[‡] Mean values within each soil type followed by the same letter are not significantly different ($P < 0.05$, using Tukey-Kramer adjustment).

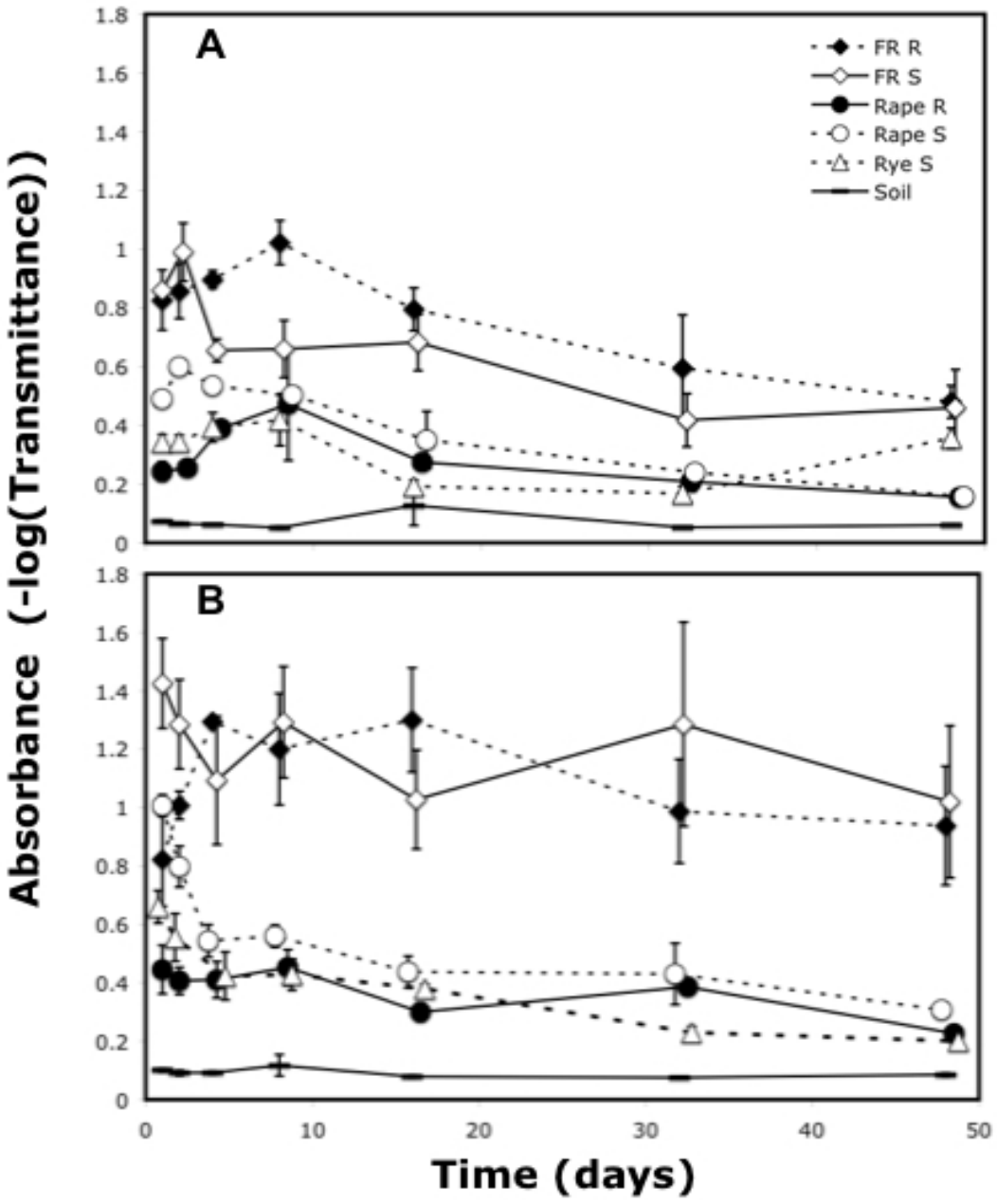

Fig. 4.5. Optical density readings of soil extracts at 410 nm in Elkton silt loam (A) and Cedartown loamy sand (B). Data points are separated horizontally for clarity. Bars are SEM, n=4 for plant amended soils; n=8 for unamended control soils.

Discussion

Plant loading rates

In our study, soils were amended with plant dry matter at a level roughly an order of magnitude greater than that used by some other incubation studies in the literature (Trintrousot et al., 2000). This loading rate could approximate decomposition dynamics from cover crop residue that is typically distributed heterogeneously in the field either upon incorporation or when left on the surface (tillage versus no-tillage management, respectively). The loading rate that we applied to small beakers of soil would be comparable to the loading rate in the top centimeter of soil in a no-tillage system in which residues are minimally incorporated. Our approach may partly address the concern of Magid et al. (2006) that the traditional incubation procedure involving homogeneous mixing of dried and milled plant materials in soil allows for reproducibility and repeatability but lacks relevance to mineralization dynamics from plant residues that are heterogeneously distributed in the field.

Carbon respiration

Our results, which show rapid initial mineralization during the first three weeks of the incubation, followed by slower rates for the duration of the incubation, are similar to results of other incubation and field studies involving soils amended with plant residues (Kirchmann and Marstorp, 1991; Magid et al., 1996; Martens et al., 2000; Trintrousot et al., 2000a and 2000b; Jensen et al., 2005; Coppens et al., 2006, Magid et al., 2006). The two phases of CO_2 evolution observed are most likely

related to initial decomposition primarily of simple carbohydrates and amino acids, followed by the decomposition of structural macromolecules such as lignin, cellulose, and phenolic compounds (Martens et al., 2000). The two-pool model we used (exponential plus linear) was appropriate for curve-fitting the C data for the duration of the study, as determined by close fits of regression lines to measured data values. Some aspect unique to tissue type besides N concentration or C/N ratios of materials that we did not study appears to have been important for decomposition of forage radish root and rye shoot, since decomposition of these materials was rapid even though these tissues had a low N concentration and relatively high C/N ratio relative to the other treatments.

The preparation of plant tissues for this study may have affected their decomposition rates, for the following reasons: First, drying plant residues at >50 °C has been shown to induce the formation of recalcitrant, lignin-like polymers in different plant materials via Maillard reactions (which can occur in the presence of heat, amino acids, water and reducing sugars) (Franzluebbers et al., 1996). Maillard reactions might occur more readily when root materials are dried, since they typically have higher concentrations of lignin and other complex C compounds than shoot materials (Franzluebbers et al., 1996). Second, milling of dried cover crop residues turned roots, particularly of forage radish, into a fine powder, while shoot materials emerged from the milling process as <1 mm flakes. While the powdery material probably bears little resemblance to substrates available to microbes in a typical field situation (Bending and Turner, 1999), increasing residue-soil contact by reducing particle size could either speed up or slow decomposition, depending on the extent to which residues are exposed to immobilizing microbes (Ambus and Jensen, 2001). Third, ground

(<1 mm) materials (including rape residues) have also been shown to become relatively protected from microbial attack as a result of increased release of polyphenolic compounds and formation of lignin-N compounds upon incorporation into soil, relative to more coarsely cut residues, although such protection is expected to be short-lived (Bending et al., 1998; Singh et al., 2006). Last, Martin (1989) observed that C mineralization from air dried roots mixed into soil was less than that of roots left to decompose in soil and reasoned that this is because microbes have to re-colonize air-dried root surfaces.

Model modification

Mathematically derived incubation parameters cannot act as stand-alone values to represent the mineralization potential of different materials (Dou et al., 1996). Pool sizes and decomposition rates have been shown to vary depending on the duration of the incubation, experimental constraints, and soil and plant physical, chemical, and biological properties (Cabrera and Kissel, 1988; Wang et al., 2004). If we assume that rates associated with the labile and recalcitrant pools do not vary for different materials, holding rate values constant while allowing pool size to vary will maximize differences among pool parameter values for different treatments when fitting curves to measured data (Wang et al., 2004). The main advantage gained in simplifying the model is that the pool sizes might better describe patterns of residue decomposition that are influenced by differences in the plant-soil mixtures. We tried two approaches in deciding on a constant value for the labile pool rate parameter. First, we compared parameter values for the different treatments after normalizing data values in terms of maximum cumulative CO_2, but found the statistical differences to be the same as for the original measured data (data not presented). In

the second approach, we held k_f constant by averaging the k_f values within each soil type, creating the following simplified equations for Elkton silt loam (2) and Cedartown loamy sand (3), respectively:

$$CO_2\text{-}C = C_f\,(1-e^{-0.138*t}) + C_s k_s t \qquad (2)$$

$$CO_2\text{-}C = C_f\,(1-e^{-0.188*t}) + C_s k_s t \qquad (3)$$

Re-running the curve-fitting program generated equations that estimated the measured data well (R^2= 0.997, data not presented). The fast pool parameter values (C_f) forage radish shoot and forage radish root were significantly greater than that for the rye shoot in the Elkton silt loam (Table 4.4). The C_f value for forage radish root was significantly greater than that for rye shoot in the Cedartown loamy sand.

Because the slow pool (Cs) and its associated rate value (ks) are not mathematically independent, a ratio can be created between the $C_s k_s$ values following "maximization of pool sizes" model simplification to compare recalcitrant pool values. As discussed by Wang et al. (2004), pool size values do not represent absolute amounts of available substrates in the short and longer-term but rather the ability for soil microbes to decompose the substrates within experimental constraints. In our study, we can infer that the slow pool of forage radish root (which had the largest $C_s k_s$ value) was the most difficult for microbes to decompose among the treatments in both

Table 4.4. Labile and recalcitrant pool equation parameters for measured CO_2 data, as generated or inferred by having constant pool rates and allowing pool size to vary.

Treatment	Plant Part	k_f	C_f	C_s*k_s	C_s pool ratio comparison[†]
			Elkton silt loam		
Forage Radish	Shoot	0.138	458 ab[‡]	1.1 a	3.6
	Root	0.138	405 ab	4.0 a	1
Rape	Shoot	0.138	416 bc	1.2 a	3.3
	Root	0.138	390 bc	2.0 a	2.0
Rye	Shoot	0.138	304 c	3.5 a	1.1
			Cedartown loamy sand		
Forage Radish	Shoot	0.188	435 b	2.6 a	1.5
	Root	0.188	488 ab	3.8 a	1
Rape	Shoot	0.188	381 ab	1.2 a	3.2
	Root	0.188	381 ab	3.3 a	1.2
Rye	Shoot	0.188	354 a	3.0 a	1.3

[†]The slow pool (C_s) can be inferred by assuming a fixed slow pool rate when creating ratios of C_sk_s for different materials, presented here as (C_sk_s of forage radish root)/(C_sk_s of other materials).

[‡]Mean values in the same column within soil type followed by the same letter are not significantly different ($P < 0.05$, using Tukey-Kramer adjustment)

soils. Materials with ratio values increasingly greater than 1 can be interpreted as being relatively easier for microbes to decompose. In the Elkton silt loam, relative ease of microbes to decompose the slow pool increased in the following order: forage radish root=rye shoot<rape root<rape shoot<forage radish shoot; while for the Cedartown loamy sand the order was forage radish root<rape root=rye shoot<forage radish shoot<rape shoot. These patterns roughly mirrored the net N mineralization/immobilization trends presented in Fig. 4.3. The obvious limitation of fixing rate constant parameters is that this may have little relation to actual rates of decomposition from labile or recalcitrant pools, which change over time with regard to their accessibility for microbial decomposition (Franzleubbers et al. 1996; Andersen and Jensen, 2001; Magid et al., 2004). Generally, however, model simplification can be useful for understanding patterns in decomposition kinetics when data for other aspects of plant residues (e.g., nitrate, lignin, cellulose, soluble and insoluble fractions content, etc.) is not gathered.

Nitrogen mineralization and immobilization dynamics

Many lab studies of N mineralization omit data representing a flush of mineral N during the first week(s) of study, considered to be the result of either rewetting dried soils and/or sieving and mixing field-moist soils (Stanford and Smith, 1972; Wang et al., 2004; Drinkwater et al., 1996). Our inclusion of N mineralization data for the first week(s) shows how relative proportions of nitrate and ammonium changed rapidly at 25 °C. Our results for most treatments suggest that nitrifying microbial populations were not limited by substrate supply, inhibited by

allelochemicals, or affected by decreases in pH that can occur upon incorporation of Brassica or rye residues into soil (Dou et al., 1996; Bending and Lincoln, 2000; Bending and Turner, 1999; Coppens et al., 2006). Nitrification did appear to be inhibited or delayed in the forage radish root and rye shoot treatments from day 4 through day 16 but our data does not allow us specifically identify the cause for this result. Treatments for which ammonium as a proportion of mineral N (Fig. 4.2) declined most rapidly (primarily the rape and forage radish shoot treatments, by day 8) exhibited most N mineralization compared to the other treatments (Fig. 4.4). We make an assumption that the lack of N mineralization (measured in our study as the mineral N concentration in soil) for treatments besides forage radish shoot and rape shoot relative to the control soil is in fact evidence of N immobilization by the microbial biomass, since C mineralization was significantly larger throughout the incubation in all treatments compared to the control soils for both soils (data not presented). Based on the finding of Recous et al. (1990) that immobilization occurs primarily from the ammonium pool, we suspect that nitrifying bacteria did not have a chance to oxidize N mineralized from residues except for in the forage radish shoot- and rape shoot-amended Elkton silt loam and in the rape shoot-amended Cedartown loamy sand.

Cumulative N mineralization appeared to follow a linear pattern, which is similar to results in other relatively short-term incubations (De Neve et al., 2004; Singh et al., 2006). Our observation of differences in N mineralization when the same tissues are used to amend contrasting soils is not uncommon (Cabrera et al., 2005). We postulate several (probably interacting) factors that are responsible for

these differences for N mineralization between the two soils types used. The Elkton silt loam has a larger (if still modest) native soil organic matter pool and a higher pH, which respectively could sustain a larger and more active microbial biomass population and provide more optimal conditions for nitrification than in the Cedartown loamy sand. In addition, the moisture content of the Elkton silt loam was less variable throughout the incubation (varying between 50-60% WFPS than the Cedartown loamy sand, which lost moisture more easily (data not presented). At day 48, WFPS in the loamy sand had dropped to 35-45%. This observation of decreased nitrification in relatively drier soils is supported by the finding of De Neve and Hofman (2002), who observed increases in net N mineralization from soils amended with carrot leaves with increases in soil water holding capacity from 18-45 %. Also, the formation of aggregates that occurred especially when mixing plant residues into the Elkton silt loam also might have allowed relatively better physical contact of residues with the soil microbial biomass compared Cedartown loamy sand, which could promote nitrification from some treatments. It is worth noting, however, that formation of aggregates could also promote the opposite effect (e.g. suppress nitrification) through stabilization of organic matter via complexes with clay and silt particles. This could partly explain why there was ultimately less cumulative N mineralization from forage radish root residues (which were added to the soil as a powder of particles with a high surface area, rather than as tissue flakes, as for shoot materials) in the Elkton silt loam as compared to that in the Cedartown loamy sand.

Comparing C and N mineralization dynamics

Adding dry matter to soils substantially increased the amount of N in soil-dry matter mixtures relative to total N in un-amended soil (Table 4.1). Relative to other studies, the N-rich plant material as we did appears to have lessened the potential for immobilization that we might have seen otherwise if we had used plant materials with wider C/N ratios (Trintrousot et al., 2000a). Our results for C and N mineralization are similar enough to others' to infer that net N mineralization relative to control soils from all residues with a C/N ratio <24 could have been expected if the length of our study had been tripled (Trintrousot et al., 2000a). In our experiment, net N immobilization (negative mineralization values relative to control soil) was mild or non-existent, which implies that soils were not N-limited and that the N added with the plant tissues was about equal to that required by the microbial biomass (Cabrera et al., 2005). Other studies have shown immediate, substantial, and persistent (lasting for several months) net immobilization following the incorporation of plant materials (even those with low C/N ratios) in N-limited soils (Jensen, 1994, Mary et al., 1996; Trintrousot et al., 2000a; Jensen et al., 2005).

The high amount of C respired from all treatments suggests that a substantial portion of the dry matter N had become assimilated by the microbial biomass by the end of the experiment. Once mineralization begins in soils from low C/N residues, this period of N release is expected to persist only briefly (i.e., for several weeks) as reported in results from field and controlled lab experiments (Thorup-Kristensen, 1994; Kirchmann and Marstorp, 1991.) Vos and van der Putten (2001) observed in a field experiment that approximately 75% of net mineralization from rape, rye, and forage radish residues (shoots and roots to 0.1 m depth soil) occurred in the 5 weeks following their incorporation in spring. The explanation for why net mineralization rates slow after a brief, intense period of mineralization is that decomposition of labile components increases the soil microbial population, which then decomposes the remaining, recalcitrant residues less quickly as the relative availability of N decreases. (Wang et al., 2004; Jensen, 1994).

We should not assume, however, that only N fractions traditionally assumed to be labile (e.g. amino acids, nitrate, etc.) were mineralized initially; Magid et al. (2004) observed that forage radish shoot dry matter decomposed rapidly (even at temperatures as cold as 3 °C) despite having a high lignin composition of 10-11%, when lignin is ordinarily expected to slow initial N mineralization. Recalling that pool sizes do not represent absolute amounts of available substrates but rather the ability for soil microbes to decompose the substrates within experimental constraints, it fits that treatments with the largest $C_s k_s$ pool size ratio, as estimated using the modified C mineralization model and as compared to the slowest-to-decompose treatment (forage radish root) had the greatest N mineralization (forage radish shoot

in the Elkton silt loam and rape shoot in Cedartown loamy sand). Finally, the relatively better adsorption of soluble C compounds (as determined by comparing the color intensity of soil extracts, Fig. 4.5) in the Elkton silt loam compared to in the Cedartown loamy sand did not seem related to N mineralization trends.

Conclusion

Variation in C mineralization from cover crop residues incorporated into soil depended partly on N concentration of materials added and partly on some physical and/or chemical aspect(s) of the plant material or of the soil-plant material mixtures that we did not measure. Nitrogen concentration of plant materials appeared to be relatively more important for the process of N mineralization from these materials than for the process of C mineralization. Nitrogen in incorporated residues induced either net N mineralization (rape and forage radish shoot), mild net immobilization (forage radish root), or no net difference in mineralization/immobilization relative to control soils (rape root, rye shoot treatments) during our short-term incubation study. Based on a comparison of slow C pool ratios, N concentrations of materials, and kill dates in the field, we can hypothesize that net N mineralization rates over the longer term (several months from killing) from the treatments studied might decrease in the following order: forage radish shoot > rape shoot > rape root >rye shoot ≥ forage radish root. A combination of factors, including plant-soil contact, soil moisture content, and differences in microbial biomass pools, may explain the somewhat different N and C mineralization trends observed in soils of different textural class. Longer-term studies of the effects on decomposition and N mineralization of using

dried versus fresh or frozen forage radish materials (because forage radish is killed by freezing in the mid-Atlantic region), and of drying and rewetting of all cover crop residues (as is likely to happen on the soil surface in a no-till system) are warranted.

Conclusions

Main finding: forage radish and rape are both well suited for use in the mid-Atlantic region as winter cover crops for the purpose of nitrogen cycling.

(1) Potential environmental advantages of growing forage radish and rape: during our study the Brassicas took up as much N from the soil profile as rye when planted in August or September.

(2) Potential agronomic advantages of growing forage radish and rape: during our study the Brassicas provided earlier release (March through May for forage radish and May-June for rape) of N to main crops in spring than rye.

Cautions: (1) Modest to moderate amounts of N from frost-killed tissues (especially from forage radish) could be lost through leaching in coarse-textured and/or well-drained soils; (2) within horticultural rotations that include Brassica main crops, the influence of planting Brassicas as winter "break" crops, in terms of fostering diseases and agricultural pests, is not known.

Additional research needs include: (1) identifying whether N turnover from Brassicas can improve N use efficiency by main crops in the longer term (full-season and multiple seasons); (2) determining the extent to which using Brassica cover crops might allow farmers in this region to decrease fertilizer application rates without affecting main crop yields; (3) further investigation of the potential of the forage radish and rye mixture to achieve N uptake in fall and release N to main crops in spring.

Appendix A: Comparison of tillage method on spring N mineralization from cover crop residues.

Introduction

The influence of spring disking versus killing with glyphosate on N mineralization from cover crop residues was investigated at CMREC in 2005. Based on the findings of Doran (1980) and others, we hypothesized that N mineralization in no-till plots would be delayed relative to that in tilled plots.

Methods

The design for the experiment was randomized complete strip-split-plot, with block*tillage type (disk or no-till)*depth (0-15 or 15-30 cm) as the sub-plot unit and a plot size of 3 x 9 m. The cover crop treatments were forage radish, rape and rye. Cover crops were broadcast seeded at CMREC using a hand-held portable spreader into standing, senescing soybeans (50% yellow, 50% green leaves) on 07 August 2004. Forage radish was killed by freezing temperatures in the third week of December 2004. In spring, selected strips in the field were either disked or sprayed with glyphosate (1.1 L ha^{-1} a.i.) on 27 April 2005, which killed the rye and rape cover crops. Soil sampling methods were the same as previously described in Chapter 3 of this thesis. Immature corn (V6) stage corn was sampled on 11 June 2005, as previously described in Chapter 3 of this thesis.

Sample codes used for analysis of this data:

title1 NO3;

proc mixed;

class rep trtname manage;

model kghaNO3 = trtname|manage/ddfm=satterth;

random rep rep*trtname rep*manage rep*trtname*manage;

lsmeans trtname*manage/adj=tukey;

run;

In the random statement:

- "rep*trtname" creates df for cover crop, [(blk -1)*(cover crop treatment-1)];
- "rep*manage" ...creates df for tillage or no tillage, [(rep-1)*(manage-1)];
- "rep*manage*trtname creates df for the interaction,

  [(rep-1)*(manage-1)*(cover crop treatment-1)]

Results

No treatment differences due to tillage method were observed either in soil or plant samples (Fig. A1 and Table A1). For soil NH_4-N concentration, there were no significant differences between cover crop treatments, or by depth, on any dates. Soil NO_3-N concentrations were significantly greater in the forage radish plots (disked and sprayed) compared to the no cover plots (disked and sprayed) in April. Soil mineral N concentrations were generally (but not significantly) greater in the 0-15 cm soil layer compared to the 15-30 cm soil layer for all dates. Immature corn plants were generally larger in disked plots compared to sprayed plots, but this difference was not significant. Corn plants in the forage radish plots were significantly larger than those in the no cover plots, and corn plants in both these treatments were significantly

larger than those in the rye plots.

Discussion and conclusion

Contrary to our expectation that tillage would speed up N mineralization rates, we saw only significant effects of cover crop treatment but not cover crop management in this experiment. Decomposition of forage radish residues, which die in winter and which decompose throughout late winter and early spring appear to be rapid regardless of whether residues are incorporated into soil or not. We suspect that immobilization in the no cover and rye plots may explain why no significant difference in N mineralization from the cover crop residues due to disking or spraying was observed.

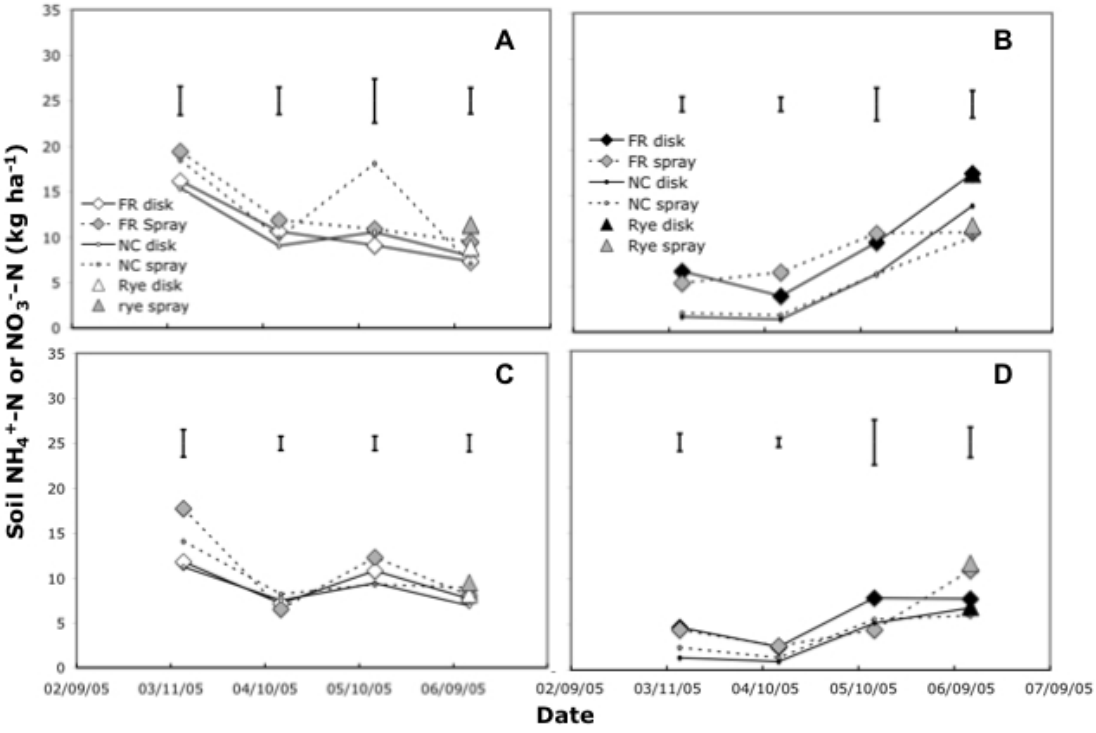

Fig. A1: Ammonium (unfilled symbols in disked plots and gray symbols in sprayed plots) (A,C) and nitrate (filled symbols in disked plots and gray symbols in sprayed plots) (B,D) at 0-15 cm (A,B) and 15-30 cm (C,D) depths at CMREC. Vertical bars are average SEM.

Table A1. Comparing the effects of tillage on N response by immature corn plants.

Cover crop treatment	Dry weight (g plant^{-1})	
	Disk	Spray
Forage radish	2.7 (0.3) †	2.3 (0.3)
Rye	0.9 (0.1)	0.7 (0.1)
No cover	2.3 (0.3)	2.0 (0.3)

† Numbers in parentheses are SEM

Appendix B: Carbon and N mineralization from rye roots incubated in Cedartown loamy sand.

Introduction

The mineralization of C and N from rye roots was compared to that from Brassica shoots and roots and rye shoots, as described in Chapter 4. This data is presented here as an appendix because the rye roots used in the experiment were planted in spring rather than in fall like the other materials.

Results

Despite having a low C/N ratio (17.4), C mineralization from rye roots was slow relative to the other treatments (Fig. B1). After a brief period of net mineralization between days 8 and 16, the amount of N turning over reached a plateau (Fig. B2).

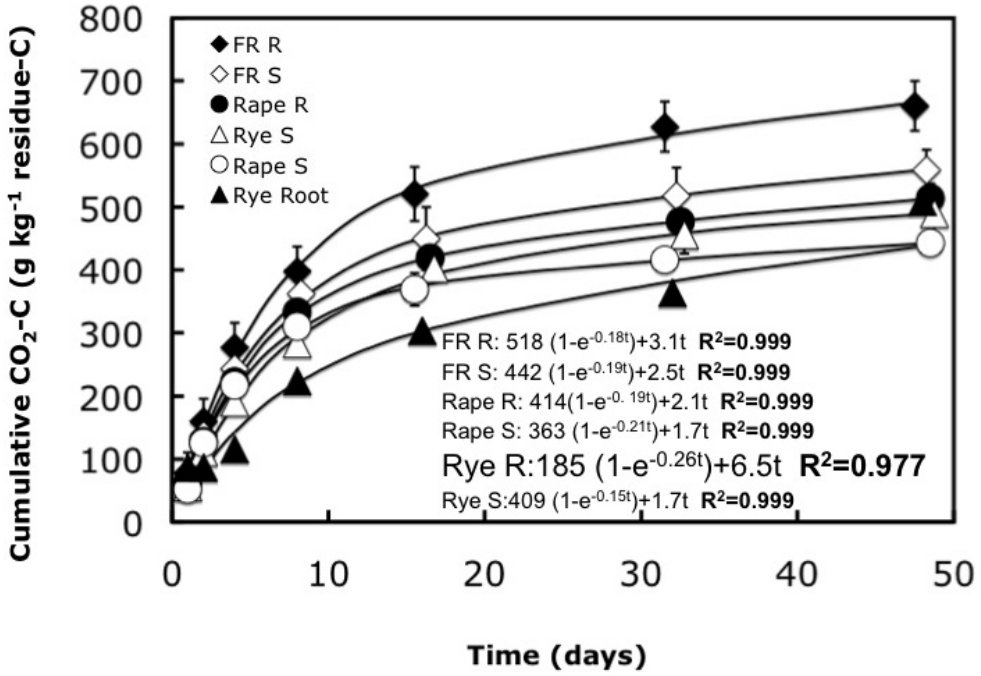

Fig. B1. Cumulative CO_2 evolution (points=measured and lines=estimated) over time. Bars are SEM, n=4. Background CO_2 and CO_2 evolved from control soil have been subtracted from data. Equations on graph present parameter constants for the two-pool model used to fit regression lines to the measured data.

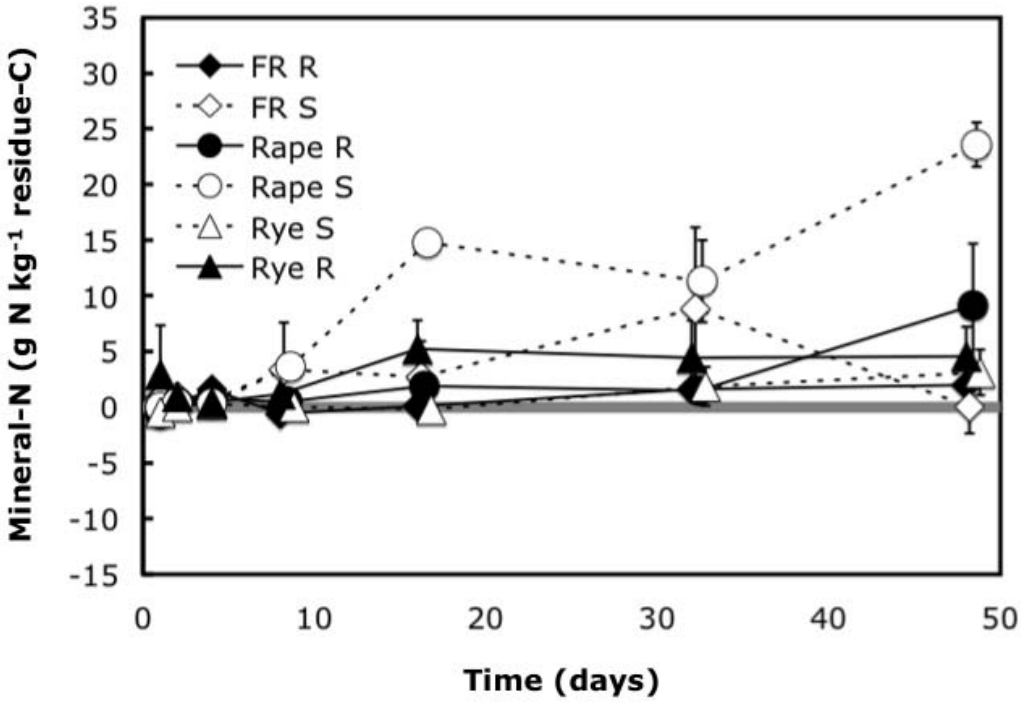

Fig B2. Mineral N (NH_4-N + NO_3-N) in amended Cedartown loamy sand. Immobilization relative to control soil values is expressed as negative values. Data points are separated horizontally for clarity. Error bars are SEM, n=4; n=8 for control soil).

Appendix C: Sample SAS codes

1. Sample SAS codes used to perform ANOVA analyses on mineral N concentration in different cover crop treatment plots, as a split plot by depth:

```
proc sort; by date;
proc mixed data=w04depth;
by date;
class rep trt depth;
model kghano3= trt|depth /ddfm=satterth;
random rep rep*depth;
lsmeans trt*depth depth/ adj=tukey;
run;
```

2. Sample SAS codes used to perform ANOVA analyses on parameter values generated for C mineralization in incubated soil follow:

(a) Comparison of a regression parameter between the two soil types used:

```
proc mixed;
class rep trtname location;
model ksXcs=trtname|location/ddfm=satterth;
random rep;
lsmeans trtname*location/ adj=tukey; run;
```

(b) Comparison of a regression parameter analyzed separately for each soil type:

```
proc sort; by location;
proc mixed; by location;
class rep trtname;
model ksXcs=trtname/ddfm=satterth;
random rep;
lsmeans trtname/ adj=tukey;
run;
```

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