ABSTRACT

Title of Thesis: FORAGE RADISH COVER CROP EFFECTS ON MYCORRHIZAL COLONIZATION AND SOIL TEST PHOSPHORUS

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Forage radish (*Raphanus sativus* L. var. *longipinnatus*) and cereal rye (*Secale cereale* L.) cover crops were examined for their effects on arbuscular mycorrhizal colonization and P acquisition of a subsequent corn (*Zea mays* L.) silage crop. Soil test P following these cover crops was also measured in bulk soil collected at three depths in the surface soil and in soil sampled within 3 cm of forage radish tap root holes. Forage radish never decreased mycorrhizal colonization and rye sometimes increased colonization of the subsequent crop compared to growing no cover crop. The extent of colonization of corn roots by arbuscular mycorrhizal fungi was positively correlated with corn shoot tissue P concentrations. Slight vertical soil test P stratification in the bulk soil occurred following both forage radish and rye cover crops at some sites. A large increase in soil test P occurred within 3 cm of forage radish tap root holes.

FORAGE RADISH COVER CROP EFFECTS ON ARBUSCULAR MYCORRHIZAL FUNGI AND SOIL TEST PHOSPHORUS

By

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Table of Contents

List of Tables

List of Figures

Chapter 1. Introduction

Background and Problem Definition

Phosphorus (P) is an essential plant macronutrient that presents unique challenges for agricultural management. In soil, P is relatively immobile due to sorption with clay minerals and metal oxides and precipitation with calcium (Ca), iron (Fe), and aluminum (Al) to form sparingly soluble salts. Due to the strong sorption to soil colloids, the rate of P diffusion towards plant roots can be slower than the rate of P uptake, and P deficient zones around plant roots can develop.

Industrialized agriculture overcomes the problem of P deficiency through the use of P fertilizers. If enough P fertilizer is added, the P sorption capacity of the soil becomes saturated and the mobility of P in the soil increases. This solution has several drawbacks, however, as excessive application of P fertilizer is a frequent contributor to water pollution (Sharpley et al., 2001; Tiessen, 1995), P fertilizers are prohibitively costly to many farmers in the developing world, and the phosphate rock from which P fertilizers are manufactured is a limited resource (Jasinski, 2006).

These concerns about the excessive use of P fertilizers have led to the study of alternative mechanisms to enhance P availability in agricultural systems. Arbuscular mycorrhizal fungi (AMF), which form a symbiotic relationship with the roots of most agricultural crops, can aid in the acquisition of existing soil P by plant roots and also improve soil structure and sequester carbon. Many species of cover crops are hosts to AMF. The use of AMF host cover crops can maintain active AMF populations between main crops, leading to rapid colonization and improved P acquisition of subsequent crops (Boswell et al., 1998; Deguchi et al., 2007).

Forage radish (*Raphanus sativus* L. var. *longipinnatus*) is being used in many parts of the world as a winter cover crop to alleviate soil compaction, reduce nitrate leaching, suppress weeds and control erosion (Weil and Kremen, 2007). Forage radish is a member of the *Brassica* family, one of the few plant families whose members do not host AMF (Ocampo et al., 1980; Vierheilig et al., 2000). Plants in the *Brassica* family contain glucosinolates which can be hydrolyzed by the enzyme myrosinase to form isothiocyanates (ITCs), chemicals that have anti-fungal properties (Schreiner and Koide, 1993a; Schreiner and Koide, 1993b; Vierheilig and Ocampo, 1990a; Vierheilig and Ocampo, 1990b; Vierheilig et al., 2000). However, microbial consumption and reactions with organic matter rapidly degrade ITCs in soil (Gardiner et al., 1999; Morra and Kirkegaard, 2002; Rumberger and Marschner, 2003).

In addition to being an AMF non-host, forage radish has other unique properties, including a relatively high tissue phosphorus (P) concentration, rapid dry matter accumulation in the fall, and rapid residue decomposition in the spring. These characteristics of forage radish cover crops present several potential opportunities and challenges for P management in the agroecosystem, including remediation of excessively high P soil, increased stratification of P at the soil surface, and increased plant available P.

Agricultural soils that are excessively high in P are common in the developed world. Phosphorus transport from such soils to natural waters is one of the primary causes of eutrophication (Boesch et al., 2001; Sharpley et al., 2001). The concentration of P in soils can be reduced over time by eliminating the use of fertilizers containing P while continuing to remove P from the soil through harvested crops (Brown, 2006;

Eghball et al., 2003). Cover crops may be harvested to feed to livestock directly as green chop or to make silage (Kratochvil et al., 2006). Harvesting a cover crop such as forage radish in addition to the main crops in a rotation could increase the amount of P removed from the soil each year, resulting in faster remediation of the soil to environmentally optimum P levels. The amount of manure or compost farmers can apply to fields is often severely restricted under P-based nutrient management plans (Brown, 2006; Kratochvil et al., 2006). In some of these cases, the additional removal of P in harvested cover crops could also allow farmers to increase the allowable manure application rates according to their nutrient management plans.

On the other hand, allowing cover crop residues to decompose at the soil surface, as is common practice in no-till agriculture, may lead to an accumulation of P in surface soil layers where it is susceptible to losses by runoff and erosion. Agricultural soils under no-till management commonly develop high P concentrations at the surface where crop residues remain and fertilizers are applied because P is relatively immobile in soil (Duiker and Beegle, 2006; Garcia et al., 2007; Sharpley, 2003; Weil et al., 1988). Cover crops that accumulate large quantities of P in their shoots may accentuate the stratification of soil P.

In soils that are low in plant available P, biological mechanisms to increase P fertility can be used when soluble P fertilizers are not accessible to farmers. Members of the *Brassicaceae* plant family can solubilize recalcitrant forms of soil P by changing the rhizosphere pH (Grinsted et al., 1982; Hedley et al., 1982; Hinsinger and Gilkes, 1997; Marschner et al., 2007) and exuding organic acids (Hoffland et al., 1989; Shahbaz et al., 2006; Zhang et al., 1997). Unfortunately, rotations or intercrops of Brassica species have

shown little effect in improving P availability for the subsequent or companion crop (Wang et al., 2007; Weil, 2000). When *Brassicas* are grown as either a cash crop or as an intercrop, the soil P mobilized by the *Brassica* crop is either removed by the harvested biomass or sequestered in plant tissue. A *Brassica* green manure or cover crop may be more effective in improving subsequent P availability because the P mobilized by the *Brassica* would be cycled back into the soil as the residues decompose. Cavigelli and Thien (2003) found that P uptake of a sorghum (*Sorghum bicolor* L.) crop was positively correlated to the P uptake of a previous perennial forage green manure crop. In low P soil, *Brassica* species may prove advantageous as green manure crops due to their ability to accumulate greater levels of tissue P.

Justification for Research

Forage radish is an increasingly used winter cover crop in the Mid-Atlantic United States. As its use expands, understanding the way in which this cover crop affects soil P and AMF will be important to maximize its benefits while minimizing any negatives. While there have been several studies on the effects of *Brassica* crops such as rape (*Brassica napus* L.) and cabbage (*Brassica oleracea* L.) on AMF, we found no studies that investigated the effect of a forage radish cover crop on AMF colonization of the next crop.

Forage radish exhibits several unique characteristics; some of which may increase negative effects on AMF and others which may decrease negative effects on AMF. First, forage radish is grown as a winter cover crop between two successive summer crops rather than as a summer crop itself. As such, the duration between the mycorrhizal host crops grown prior to and following a forage radish cover crop is much shorter (~6

months) than the duration between mycorrhizal host crops when a *Brassica* is grown as a summer cash crop (>1 year). The hyphal networks of AMF that are established during the growth of a host crop can retain their colonization potential in undisturbed soil from fall to spring but the colonization potential decreases as the length of time without a host crop increases (Kabir et al., 1999; McGonigle and Miller, 1999).

The second difference between the potential effect of forage radish cover crops and other *Brassica* crops on AMF is the timing of the release of ITCs. When planted in fall as a cover crop, forage radish is killed at a vegetative growth stage when winter temperatures fall below -4 ^{o}C. Following its death, glucosinolates in the forage radish tissue are converted into ITCs and are released into the soil and atmosphere. Other *Brassica* crops, such as canola (*Brassica napus* L.), often aren't killed until after reproductive maturity is reached. Tissue concentrations of glucosinolates, the precursor compounds to ITCs, are at their highest during vegetative growth stages, decline after flowering, and are absent by senescence (Kirkegaard et al., 2000). On the other hand, ITC toxicity to soil organisms is reduced at low temperatures (Matthiessen and Shackleton, 2005), such as would occur when forage radish cover crops winter-kill and presumably release ITCs into the soil.

The effect that forage radish cover crops have on the stratification of P in no-till agriculture is also unknown. In soils that are already high in P, increased stratification of P at the surface could be undesirable because of increased susceptibility to losses by run-off and erosion. In low P soils, though, forage radish may be able to concentrate P at the soil surface and in proximity to its taproot holes, increasing P availability for subsequent crops.

General Research Approach

Field experiments were conducted at three sites near Beltsville, MD. The experiments lasted two complete years at each site, starting in August 2006 and ending in August 2008. At all sites a randomized complete block experimental design with four replicates was used. There were three cover crop treatments common to all sites: forage radish, cereal rye (*Secale cereale* L. cv. 'Wheeler'), and no cover crop. At one site, a fourth cover crop treatment was included: a rye/radish mixture with alternating rows of rye and radish.

At all sites, forage radish cover crops were frost-killed during January and cereal rye cover crops were killed with herbicides in the spring prior to corn planting. Following termination of the cover crops, corn (*Zea mays* L.) was planted in the spring and whole plants were harvested in mid-August for silage. No-till practices were used for the duration of the experiment to minimize soil disturbance, which can negatively affect AMF. Phosphorus fertilizers were not used during the experiment to maximize the effect of AMF on corn growth.

Cover crop tissue samples were taken in the late fall, near the time of maximum dry matter accumulation of forage radish, and again in the spring for rye, prior to killing it with herbicides. Dry matter production and tissue P concentrations were measured to determine total P uptake by the cover crop treatments.

Soil samples were taken each spring at the time of corn planting at three depth ranges: 0 to 2.5 cm, 2.5 to 10 cm, and 10 to 20 cm. Mehlich 3 soil test P was measured on each soil sample to determine the effects of the cover crops on P stratification. At one site in the spring of 2008, small scale sampling of the soil around forage radish tap root

holes was conducted to determine soil test P levels in proximity to forage radish root holes.

Corn roots were sampled at the V4 and V8 growth stages (McWilliams et al., 1999) to measure colonization by AMF. Corn shoots were sampled at the V4, V8, and R1 stages to measure dry matter production and P concentration. Corn silage yield was also measured.

General Research Questions and Objectives

Question 1: Do forage radish, rye, and a mixture of forage radish and rye cover crops affect mycorrhizal colonization of the subsequent crop?

Hypotheses: Forage radish will decrease mycorrhizal colonization of the next crop compared to no cover crop. Rye and a mixture of rye and radish will increase mycorrhizal colonization compared to no cover crop.

Question 2: Do forage radish, rye, and a mixture of forage radish and rye cover crops affect P acquisition of the subsequent crop?

Hypothesis: Forage radish, rye, and a mixture of radish and rye all will increase P acquisition of the next crop compared to no cover crop.

Question 3: Do forage radish and rye cover crops take up different quantities of P?

Hypothesis: Forage radish will take up a larger quantity of P than rye.

Question 4: Will forage radish and rye cover crops increase soil test P at the soil surface and near root holes?

Hypothesis: Both forage radish and rye cover crops will increase soil test P at the surface, but forage radish more so than rye. Soil test P will be greater in the vicinity of radish root holes.

Colonization of corn roots by AMF will be measured following the cover crop treatments to determine if forage radish has a negative effect or if rye has a positive effect on AMF colonization potential. Corn roots will be measured for AMF colonization at two growth stages, V4 and V8, to determine if cover crops effects on AMF colonization are temporary or longer-lived. Corn shoot dry matter production and P concentration will be measured at the V4, V8, and R1 growth stages to determine if AMF colonization of corn roots is associated with P acquisition and growth of corn plants. Corn silage yield will also be measured to determine if AMF colonization and P acquisition are associated with yields.

Cover crop dry matter production and tissue P concentration will be measured to determine the potential of forage radish and rye cover crops to acquire P from the soil. Soil test P at 0 to 2.5 cm, 2.5 to10 cm, and 10 to 20 cm will be measured following cover crop treatments to determine if cover crops result in P stratification. Soil test P around radish root holes will also be measured to determine if forage radish enriches plant available P near its root holes.

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Chapter 2. Literature Review: Effects of Mycorrhizal Host and *Brassica* Non-Host Cover Crops on Mycorrhizal Fungi and Phosphorus Availability

Introduction

Phosphorus (P) is an essential plant macronutrient that presents unique challenges for agricultural management. In soil, P is relatively immobile due to sorption with clay minerals and metal oxides and precipitation with calcium (Ca), iron (Fe), and aluminum (Al) to form sparingly soluble salts. Due to the strong sorption to soil colloids, the rate of P diffusion towards plant roots can be slower than the rate of P uptake, and P deficient zones around plant roots can develop.

Industrialized agriculture overcomes the problem of P deficiency through the use of P fertilizers. If enough P fertilizer is added, the P sorption capacity of the soil becomes saturated and the mobility of P in the soil increases. This solution has several drawbacks, however, as excessive application of P fertilizer is a frequent contributor to water pollution (Sharpley et al., 2001; Tiessen, 1995), P fertilizers are prohibitively costly to many farmers in the developing world, and the phosphate rock from which P fertilizers are manufactured is a limited resource (Jasinski, 2006).

These concerns about the excessive use of P fertilizers have led to the study of alternative mechanisms to enhance P availability in agricultural systems. Arbuscular mycorrhizal fungi (AMF), which form a symbiotic relationship with the roots of most agricultural crops, can aid in the acquisition of existing soil P by plant roots and also improve soil structure and sequester carbon. Many species of cover crops, are hosts to AMF. The use of AMF host cover crops can maintain active AMF populations between

main crops, leading to rapid colonization and improved P acquisition of subsequent crops (Boswell et al., 1998; Deguchi et al., 2007).

Brassica cover crops, which are AMF non-hosts, are being used by increasing numbers of farmers in the Mid-Atlantic United States (Weil and Kremen, 2007). In addition to being AMF non-hosts, *Brassicas* release anti-fungal isothiocyanates (ITCs) upon decomposition. The effect of *Brassica* crops on AMF has been studied, with some studies finding a negative effect (Gavito and Miller, 1998b; Sorensen et al., 2005) and other studies no effect at all (Ocampo and Hayman, 1981; Ryan and Angus, 2003).

Although *Brassicas* are AMF non-hosts, they have other mechanisms for increasing plant available P in the soil such as changing the rhizosphere pH and exuding organic acids (Hinsinger and Gilkes, 1997; Hoffland, 1992). Cover crops in general can also increase plant available P by adding organic matter to the soil, which blocks P sorption sites and releases orthophosphate through mineralization (Horst et al., 2001).

This literature review will focus on the use of cover crops to promote AMF, the effect of *Brassica* crops on AMF, and the use of plant based mechanisms to increase plant available P.

The Role of AMF in P Acquisition by Plants

The mycorrhizal association between plant roots and AMF is symbiotic; an exchange of nutrients and energy occurs between the two organisms (Brundrett, 2004). Arbuscular mycorrhizal fungi colonize plant roots and receive plant photosynthate as an energy source while providing phosphorus (P) and zinc (Zn) from the soil to the plant (Cooper and Tinker, 1978; Hamilton et al., 1993). The majority of AMF species are

obligate symbionts, meaning they cannot survive over extended time periods in the absence of a host plant to provide them an energy source (Brundrett, 2002).

Due to the slow rate of diffusion of P in the soil, P deficient zones can form in the soil around plant roots. The external hyphae of AMF can grow beyond these P deficient zones, absorb P from the soil and transfer it to plant roots over a distance of up to 7 cm (Rhodes and Gerdemann, 1975), thereby enlarging the soil volume accessible to plants for nutrient acquisition. Arbuscular mycorrhizal fungi can transport P through their hyphae unimpeded by the sorption to clay and oxide surfaces which normally limits the rate of P diffusion in soil. This results in greater rates of P uptake in plants colonized by AMF than in un-colonized plants (Smith, 1982).

Numerous field studies have demonstrated the benefits of the mycorrhizal association to agricultural crops, especially at early growth stages and in soil low in plant available P. Root colonization of corn (*Zea mays* L.) and AMF hyphal density in soil at early growth stages (<V6 stage) were positively correlated with P uptake, which in turn was positively correlated with yield in studies conducted by Deguchi et al. (2007), Boswell et al.(1998), Kabir and Koide (2002), and Gavito and Miller (1998a) in P deficient soil. Increased colonization levels have also resulted in decreased time to reproductive maturity in corn and soybeans (*Glycine max* L.) (Deguchi et al., 2007; Goss and de Varennes, 2002). In soil with adequate plant available P, increased mycorrhizal colonization has generally not translated into increased yield or growth (Galvez et al., 2001; McGonigle and Miller, 1993; Sorensen et al., 2005).

Cover Crop Effects on AMF

Cover crops are important components of agricultural systems due to the many environmental and agronomic benefits they provide. Cover crops can reduce leaching of nutrients, reduce surface runoff and soil erosion and increase levels of soil organic matter (Fageria et al., 2005). Many cover crop species, such as wheat (*Triticum aestivum* L.), cereal rye (*Secale cereale* L.), oats (*Avena sativa* L.), and clover (*Trifolium sp.*) host AMF. By hosting AMF through the winter, these cover crops allow AMF to maintain an active hyphal network in the soil, which leads to increased colonization and improved P nutrition of the subsequent crop (Boswell et al., 1998; Deguchi et al., 2007; Galvez et al., 1995; Kabir and Koide, 2002; Karasawa et al., 2001; Sorensen et al., 2005).

Cover crops that host AMF may be particularly important in maintaining mycorrhizal activity in tilled soil. Tillage destroys the external hyphae of AMF, rendering it unable to colonize subsequent plant roots (Evans and Miller, 1990; Jasper et al., 1989). However, AMF hyphae that are associated with root fragments retain some degree of their colonization potential following a tillage event (Jasper et al., 1989). Boswell et al. (1998), found that AMF colonization of corn following tillage was higher when a winter wheat cover crop was grown than when no cover crop was grown.

Sorensen et al. (2005) compared the effect of fall or spring tillage of a black medic (*Medicago lupulina* L.) cover crop on subsequent AMF colonization of leeks (*Allium ampeloprasum* L.). There was no difference in leek root colonization between the two dates of cover crop incorporation and both cover crop treatments resulted in greater leek root colonization than a tilled no cover crop control treatment. Furthermore, cover crop treatments that were killed in the spring without soil disturbance had equal levels of colonization as the tilled cover crop treatments. Root segments of the tilled

cover crops acted as sufficient inoculum to overcome the loss of the intact external hyphae as a source of inoculum. However, the experiment was conducted in a high P soil and effects of cover cropping on mycorrhizal colonization did not translate into changes in P uptake or shoot growth.

Kabir and Koide (2002) discussed other possible explanations for improved P uptake following cover cropping. One factor is the decomposition of cover crop biomass, which can mineralize P from the tissue and release organic acids that make P more plant available in soil. Cover crops can also improve soil aggregation influencing root growth, microbial activity, and nutrient uptake. Despite these other possible explanations, the significant correlations between measurements of AMF and measurements of plant P and yield in most cover crop research provides the most evidence towards a mycorrhizally mediated effect.

Toxicity of *Brassica* Crops to AMF

The *Brassicaceae* is one of the few plant families whose members do not host AMF (Ocampo et al., 1980; Vierheilig et al., 2000). One proposed reason for the non-mycorrhizal status of the *Brassicaceae* is that their tissues contain glucosinolates, which can be hydrolyzed by the enzyme myrosinase to form isothiocyanates (ITCs) with broad-spectrum biocidal properties (Schreiner and Koide, 1993a; Schreiner and Koide, 1993b; Vierheilig and Ocampo, 1990a; Vierheilig and Ocampo, 1990b; Vierheilig et al., 2000). Laboratory experiments have demonstrated that extracts of *Brassica* tissues and pure ITCs inhibit AMF spore germination (Schreiner and Koide, 1993a; Vierheilig and Ocampo, 1990a; Vierheilig and Ocampo, 1990b) and in field experiments, rotations of

Brassica crops have led to reductions in AMF colonization of subsequent crops (Black and Tinker, 1979; Gavito and Miller, 1998b; Sorensen et al., 2005).

One conclusion from these results is that due to the production of ITCs, *Brassicas* are toxic to AMF. This conclusion has many shortcomings, however. The ITCs produced in natural soils are subject to biological and chemical interactions within the soil ecosystem which may reduce their toxicity to soil organisms (Rumberger and Marschner, 2003; Warton et al., 2003) and these interactions are not captured by *in vitro* studies. Also, the field studies that report a reduction in AMF colonization following a *Brassica* crop were based on a comparison to AMF colonization following a host crop (Gavito and Miller, 1998b; Sorensen et al., 2005). With such a comparison, it is not possible to infer that the *Brassicas* are antagonistic to AMF. Rather, the reduction could be due to the fact AMF are obligate symbionts, and that without a host plant to provide an energy source, the inoculum potential of the AMF population will naturally decline over time (Thompson, 1987).

Glucosinolates and Isothiocyanates Produced by *Brassicas*

Glucosinolates are a category of sulfur containing compounds found in the tissue of *Brassicas* and other plant families in the order *Capparales* (Rosa and Rodrigues, 1999). Glucosinolates vary in chemical structure and can be aliphatic, aromatic, or indolyl in structure (Figure 1). Approximately 120 different glucosinolates have been described (Halkier and Gershenzon, 2006).

When plant cells are damaged, the enzyme myrosinase (β-thioglucosidase), which is normally sequestered in a vacuole within myrosin cells, will hydrolyze glucosinolates into a variety of possible products depending on environmental conditions and the

presence of co-factors. The most common hydrolysis products are ITCs, but nitriles, epithionitriles, and thiocyanates are also possible (Halkier and Gershenzon, 2006). Isothiocyanates are distinguished by the nature of the R group which is maintained from the original glucosinolate molecule. The glucosinolate hydrolysis products, especially ITCs, are the compounds with bioactive properties as opposed to the glucosinolate precursors themselves (Brown and Morra, 1997). Biologically produced ITCs share a common structure with the active product of the synthetic soil fumigant metam-sodium (sodium *N*-methyldithiocarbamate), which upon contact with a moist soil will react to form methyl isothiocyanate (Matthiessen and Kirkegaard, 2006). Halkier and Gershenzon (2006) provided a complete review of the biochemical synthesis and hydrolysis of glucosinolates in plants.

The specific glucosinolates contained within *Brassicas* vary by species, but aliphatic glucosinolates generally predominate in the shoots while aromatic glucosinolates predominate in the roots (Gardiner et al., 1999; Kirkegaard and Sarwar, 1998; Vierheilig et al., 2000). The ITCs derived from these two categories of glucosinolates differ in their volatility, with aliphatic ITCs being more volatile than aromatic ITCs (Sarwar et al., 1998). Evolutionarily, *Brassicas* may have developed glucosinolates in order to ward off pests and diseases, and the distribution of these different forms of glucosinolates between roots and shoots makes sense ecologically. Volatile ITCs formed from aliphatic glucosinolates will diffuse faster through air in above ground plant parts, while non-volatile ITCs formed from aromatic glucosinolates will diffuse faster through moist soil as a soluble compound.

Production and Degradation of *Brassica* Derived ITCs in Soil

Concentrations of ITCs released in the rhizosphere soil of living *Brassica* roots are generally in the range of 0.2 to 1 nmol/g, but concentrations as high as 10 nmol/g have been observed in the rhizosphere of canola (*Brassica napus* L.) that had been attacked by insects. Concentrations in the bulk soil under a living *Brassica* crop are generally less than 0.1 nmol/g (Rumberger and Marschner, 2003; Rumberger and Marschner, 2004).

Mechanical incorporation of *Brassica* residues into soil can result in a wide range of ITC concentrations in the soil depending on the method of incorporation, pretreatment of the residue to enhance cell disruption, soil water content, and rate of ITC degradation. Without any pretreatment, the incorporation of *Brassica* residues into a relatively dry soil results in a concentration of ITCs around 1 nmol/g (Gardiner et al., 1999; Gimsing and Kirkegaard, 2006; Morra and Kirkegaard, 2002). Morra and Kirkegaard (2002) calculated that without any pretreatment of the residue, only 1% of the glucosinolates contained in the plant matter were readily converted to ITCs following incorporation. Freezing or macerating the tissue prior to incorporation and irrigating the soil to a waterlogged condition increases ITC concentrations ten-fold on average and concentrations as high as 90 nmol/g have been observed (Gimsing and Kirkegaard, 2006; Morra and Kirkegaard, 2002). Waterlogged soils increase the concentration of ITCs because water is a reactant in the hydrolysis of glucosinolates and because in waterlogged soil the diffusion of volatile ITCs out of the soil is slower (Morra and Kirkegaard, 2002). In comparison to these concentrations of biologically derived ITCs, application of metam-sodium at recommended rates will result in ITC concentrations between 500 and 1300 nmol/g of methyl ITC (Gardiner et al., 1999).

Isothiocyanates, once produced by the hydrolysis of glucosinolates, are quickly degraded through microbial consumption and reactions with soil organic matter. When 2-phenylethyl ITC (PEITC) was added to fresh soil as a single dose at a concentration of 3.4nmol/g, it was reduced to 1.7 nmol/g after 1 hour and to 0.3 nmol/g after 21 hours. Beyond 44 hours, only traces of the ITC were recovered from the soil. When the soil was sterilized, the same initial ITC concentration added was reduced to 1.7 nmol/g after 1 hour, followed by a linear decrease to 1.1 nmol/g after 91 hours. This demonstrates that there is an initial and immediate degradation of ITCs through a reaction with soil physical or chemical components and that biological degradation will continue to rapidly deplete ITCs (Rumberger and Marschner, 2003). Following incorporation of *Brassica* residues to soil, ITC concentrations tend to peak within 24 hours, followed by a rapid decline in concentration below 1 nmol/g, even when means have been taken to maximize the release of ITCs in soil (Gardiner et al., 1999; Morra and Kirkegaard, 2002).

Rapid biodegradation of ITCs, leading to reduced efficacy at controlling diseases and pests, has been well documented within the realm of biofumigation research (Matthiessen and Kirkegaard, 2006; Warton et al., 2003). Gram-positive bacteria capable of growing on agar with methyl ITC as a carbon substrate have been cultured from soils with a history of metam-sodium application and soil inoculated with theses bacteria showed enhanced degradation of methyl ITC (Warton et al., 2001). Cold temperatures and high organic matter contents also reduce the efficacy of biologically derived ITCs at controlling insect pests in soil (Matthiessen and Shackleton, 2005).

Toxicity of *Brassicas* and ITCs to Arbuscular Mycorrhizal Fungi

Although the use of *Brassicas* as biofumigant crops has been suggested as a way to biologically control soil-borne diseases and pests, disruption of beneficial microbial activity, such as mycorrhizal symbioses could be a negative outcome of such a practice. Only one growth chamber study has been conducted on the effects of *Brassica* tissues incorporated into soil on AMF, and the results showed no effect on subsequent colonization of a host crop (Pellerin et al., 2007). In the field, interactions between tillage practices (which are a necessary component of most biofumigation procedures), soil phosphorus levels (which are likely to vary greatly between low-input and high-input farms), and the mycorrhizal dependence of subsequent crops may be important factors in the overall effect of biofumigation on the mycorrhizal symbiosis.

Little is known about the direct mechanism of toxicity of ITCs, other than that they react with amino and sulfhydryl groups of proteins (Halkier and Gershenzon, 2006), and may therefore disrupt many aspects of organismal biology, such as enzyme systems and cell wall integrity. Isothiocyanates are especially active against fungi and insects (Brown and Morra, 1997; Sarwar et al., 1998), and it should be noted that these groups of organisms contain chitin, a polymer formed from units of N-acetylglucosamine, in their cell walls and exoskeletons, respectively. The reactivity of ITCs with amino and sulfhydryl groups, which are common functional groups found in soil organic matter, may also explain the propensity of ITCs to be degraded by soil organic matter (Matthiessen and Shackleton, 2005).

Isothiocyanates are more toxic to eukarya than to bacteria and have a wide range of toxicity to fungi other than AMF (Fan et al., 2008; Smith and Kirkegaard, 2002). *Pythium irregulare*, a fungus-like disease, is notably resistant to ITCs, possibly because it

is a member of the class Oomycetes (Sarwar et al., 1998). Oomycetes, which were once mistakenly classified as true fungi, have cell walls composed of cellulose rather than chitin.

The effects of *Brassicas* and ITCs on AMF have been studied at a variety of different combinations of AMF and *Brassica* growth stages, both *in vitro* and *in vivo*, and at laboratory, growth chamber, and field scales, with results depending on the methods of the experiment.

Exposing AMF spores to *Brassica* root extracts *in vitro* reduced germination of the spores exposed to soluble ITCs and completely inhibited germination of spores exposed to volatile ITCs after 7 days (Vierheilig and Ocampo, 1990a; Vierheilig and Ocampo, 1990b). Schreiner and Koide (1993a; 1993b) observed that living *Brassica* roots in sterilized soil, or *Brassica* root extracts in fresh soil, reduced germination of AMF spores after 5 and 7 days, but after 12 or 14 days, germination had returned to control levels, suggesting that ITCs may be fungistatic in their effect on AMF spores.

When an AMF hypha grew towards and touched the root of a *Brassica* plant *in vitro*, the hyphal cells became vacuolated and retracted their cytoplasm (Glenn et al., 1985). However, there are many more reports that AMF hyphae grow normally and proliferate around and on the surface of live *Brassica* roots, especially when the root of a host plant is also present (Giovannetti et al., 1993; Glenn et al., 1988; Kabir et al., 1996; Ocampo et al., 1980). In addition, a functional symbiosis between a host plant root and AMF does not seem to be inhibited by the presence of a nearby living *Brassica* root (El-Atrach et al., 1989; Fontenla et al., 1999; Ocampo et al., 1980). In one treatment combination of the experiments by El-Atrach et al. (1989), where AMF were inoculated

as spores into a mixture of sand and vermiculite, the presence of live *Brassica* roots reduced the percentage of root length of the host plant colonized by AMF.

Other studies have investigated AMF colonization and inoculum potential following a *Brassica* crop in rotation. Before drawing conclusions from these types of studies, however, it is important to identify the treatment against which comparisons are made. For example, Gavito and Miller (1998b) found that canola reduced AMF colonization of a subsequent corn crop during the first 40 days of growth and attributed the effect to the toxic ITCs released by the canola. However, their conclusions were based on comparing a non-mycorrhizal pre-crop of canola to a mycorrhizal pre-crop of corn. Under such a comparison it is impossible to identify if the reduction in AMF inoculum potential and colonization was simply due to natural atrophy in the AMF population because of the lack of a host crop, or if the canola was actively toxic to the AMF due to the release of ITCs. Sorensen et al. (2005) found similarly that a fall cabbage (*Brassica oleracea* L.) crop caused a decrease in AMF colonization of spring planted leeks during the first 40 days of growth. However, this is also in comparison to a mycorrhizal pre-crop, rather than a bare-fallow treatment. The use of a bare-fallow treatment as a control against which comparisons can be made is the best method to make inferences about whether *Brassicas* are actively toxic against AMF beyond the fact that they do not serve as hosts.

Sorensen et al. (2005) found that the early-season reduction in mycorrhizal colonization of leeks following cabbage did not negatively affect P uptake or final yields, but the study took place soil with optimum levels of P. In a low P soil, Gavito and Miller

(1998a) found that the early-season reduction in mycorrhizal colonization of corn following canola did reduce the P uptake and harvest index of the corn crop.

Studies of the effects of *Brassica* rotations on colonization of subsequent host crops that have used a bare-fallow treatment as a control have shown that *Brassica* crops do not reduce colonization of subsequent crops any more so than a bare-fallow treatment (Ocampo and Hayman, 1981; Ryan and Angus, 2003). One study did show a reduction in AMF inoculum potential and colonization following a *Brassica* pre-crop as compared to a bare-fallow treatment, but the study was conducted in a potted mixture of sand and vermiculite (Fontenla et al., 1999), which would not degrade ITCs as readily as a natural soil would.

The bulk of research on the effects of *Brassicas* on AMF suggests that *Brassicas* in crop rotations are not toxic to AMF. Rather observed reductions in AMF inoculum potential and colonization following *Brassica* crops are likely to be a result of the natural atrophy that occurs to AMF when a suitable host is lacking. In a related area of inquiry, Smith et al. (2004) found that reductions in take-all disease (*Gaeumannomyces graminis* var. *tritici*) in a wheat crop following a *Brassica* biofumigant crop were similar to reductions in the disease which followed a bare-fallow treatment or rotations of several other non-hosts of the disease that do not produce ITCs. They concluded that the mechanism of the disease suppression could not be attributed to the biofumigant effect of *Brassica* derived ITCs as was previously thought, but was more likely due to a break crop effect where the population of the disease organism naturally declined after an extended period without a suitable host.

The lack of toxicity of *Brassicas* towards AMF could be because of the relatively low concentrations of ITCs produced by *Brassica* crops not specifically managed for biofumigation. Concentrations of ITCs released into the rhizosphere of *Brassica* roots range from 0.2 to 1 nmol/g and are less than 0.1 nmol/g in the bulk soil, which is 2 to 3 orders of magnitude smaller than the concentrations that result from recommended biofumigation practices or metam-sodium application rates (Gardiner et al., 1999; Gimsing and Kirkegaard, 2006; Morra and Kirkegaard, 2002; Rumberger and Marschner, 2003; Rumberger and Marschner, 2004). Little is known regarding the concentrations of ITCs in soils that must be reached to achieve fungal toxicity, and it is difficult to experimentally determine such levels for fungi because ITCs may affect resting spores differently than the mycelium (Matthiessen and Shackleton, 2005). However, the concentrations of ITCs that result from *Brassica* crops under normal cropping practices are much lower than the concentrations of ITCs that must be added to soil to achieve toxicity to other organisms (Matthiessen and Shackleton, 2005).

Rapid degradation of ITCs in natural soils is also likely to reduce the *in vivo* toxicity of *Brassicas* to AMF, even when high concentrations of ITCs are initially produced. Enhanced biodegradation in soil has led to a reduction in the toxicity of ITCs to other soil organisms (Matthiessen and Shackleton, 2005; Warton et al., 2003). In fact, studies that demonstrate toxic effects of *Brassicas* on AMF have usually been conducted *in vitro* (Glenn et al., 1985; Vierheilig and Ocampo, 1990a; Vierheilig and Ocampo, 1990b), in sand-vermiculite mixtures (El-Atrach et al., 1989; Fontenla et al., 1999), or in sterilized soil (Schreiner and Koide, 1993a), all conditions that would reduce the degradation rate of ITCs.

There may also be interactions between agricultural management practices and the toxicity of *Brassicas* to AMF. To achieve concentrations of ITCs in the range that are toxic to soil organisms requires incorporating *Brassica* tissues via tillage (Morra and Kirkegaard, 2002), but tillage itself also has a negative effect on the AMF mycelium and its inoculum potential (Evans and Miller, 1990). Tillage following a *Brassica* crop would probably reduce the AMF inoculum potential more so than tillage following a host crop because colonized root fragments of a prior host crop serve as important inoculum sources following tillage (Evans and Miller, 1990; Jasper et al., 1989). Concentrations of glucosinolates in *Brassicas* decline after flowering and are absent at senescence (Kirkegaard et al., 2000), so *Brassicas* that are terminated in early growth stages, such as those grown as cover crops or green manures, could potentially release more ITCs than those *Brassicas* which are grown to maturity. Finally, ITCs are more toxic at warm soil temperatures (Matthiessen and Shackleton, 2005), so the toxicity of a *Brassica* crop may be dependent on the season in which it is grown. Kirkegaard et al. (2000) also reported that a variety of environmental factors interacted with the ability of a *Brassica* biofumigant crop to reduce take-all disease.

Reasons for the Non-Mycorrhizal Status of *Brassicas*

Research on the relations between *Brassicas* and AMF have generated two main hypotheses as to why *Brassicas* are non-hosts to AMF. One hypothesis is that *Brassicas* lack a mechanism for signaling AMF, a process which is required to form a functional symbiosis (Giovannetti et al., 1993; Ocampo et al., 1980). The second hypothesis is that the presence of ITC precursors in *Brassica* roots inhibits colonization by AMF (Schreiner and Koide, 1993a; Schreiner and Koide, 1993b; Vierheilig et al., 2000). Vierheilig et al.

(2000) suggested that 2-phenylethyl glucosinolate may play a key role in the non-mycorrhizal status of *Brassicas* because it was found in large quantities in the roots of *Brassica* species, but was not found in the roots of several members of the glucosinolate containing Tropaeolaceae and Caricaceae families which do host AMF. However, other differences may exist between these plant families and correlation cannot be used to infer causality.

The recent discovery of plant produced strigolactones, which act as a signal to regulate the symbiosis between plants and AMF (Steinkellner et al., 2007; Yoneyama et al., 2008), provides a new lens through which the mycorrhizal status of *Brassicas* could be investigated. To date, no published studies have investigated the presence or absence of strigolactone production by *Brassica* species. However, Yoneyama et al. (2008) found that white lupine (*Lupinus albus* L.), an AMF non-host, did produce several types of strigolactones, but at 1/1000 the level of other AMF hosts. Additionally, while AMF host plants increased exudation of strigolactones under P deficient conditions (Yoneyama et al., 2007a; Yoneyama et al., 2007b), white lupine decreased strigolactone exudation under P deficiency.

Plant-Based Mechanisms to Increase P Availability

Increasing native soil P availability and phosphate rock availability via plant-based mechanisms has been widely studied as a means of improving soil fertility when soluble P fertilizers are not accessible to farmers. Addition of green manures, the use of cover crops, intercropping with P efficient plants, and certain crop rotations have all been shown to increase P uptake and yield, though effects are often specific to certain crops

and soils (Bah et al., 2006; Cavigelli and Thien, 2003; Pypers et al., 2007; Wang et al., 2007).

These plant-based techniques are thought to improve P availability in a number of ways: by changing the rhizosphere pH; through exudation of organic acids by roots and decomposing residues which fill P sorption sites and chelate cations which can precipitate with phosphate; and through the mineralization and uptake of P from decomposing plant residues (Hinsinger, 2001; Hoffland, 1992; Horst et al., 2001).

Bah et al. (2006) showed that incorporation of green manures increased the P uptake of a subsequent grass crop. However, isotopic labeling indicated that only 0.5% of the P acquired by the grass crop was obtained from P released by the green manures. Virtually all of the P acquired by the plant was from either native soil P or from phosphate rock amendments. An associated study showed that addition of green manures reduced the P sorption of the soil (Bah et al., 2003), and the authors suggested that the green manures increased P availability by saturating P sorption sites on Fe and Al oxides with organic ligands and phosphate ions released by decomposition. Reddy et al. (2005) also demonstrated a reduction in P sorption and an increase in bioavailable P following incorporation of soybean and wheat residue. Randhawa et al. (2005) showed that soil which was amended with a lupin (*Lupinus angustifolius* L.) green manure had greater soil solution P and exchangeable P concentrations and had greater organic P mineralization rates than unamended soil.

Certain species of plants are known to be P efficient in that they have greater P accumulation and yield than other species at low soil P levels. Some of these species include buckwheat (*Fagopyrum esculentum* L.), rape (*Brassica napus* L.), chickpea

(*Cicer arietinum* L.), pigeonpea (*Cajanus cajan* L.), and white lupin (Miyasaka and Habte, 2001). Additionally, within many plant species, some cultivars are known to be more P efficient than others. Much of the work on improving P availability via plant-based mechanisms has focused on the use of these P efficient species in crop rotations and intercropping. This review will focus primarily on work related to members of the *Brassica* family.

The two main features of *Brassica* species which allow them to access soil P unavailable to many other plant species are their ability to change the rhizosphere pH and to exude large quantities of organic acids from their roots. Grinsted et al. (1982) found that rape reduced the pH of rhizosphere soil from 6.5 to 4.1, and that the acidification of the rhizosphere only occurred after roots began competing with each other for soil P. Phosphorus desorption isotherms across a range of soil pH showed that 10 times as much P could be desorbed from the soil at pH of 4.5 compared to pH 6.2. Hedley et al. (1982) determined that the pH change caused by the rape was due to an imbalance of cation and anion uptake created by a steady decline in NO_3^- uptake and a steady increase in Ca^{2+}. This imbalance was counteracted by the exudation of H^+ ions by the roots. There was no difference in extractable organic acid concentrations or acid producing microorganisms between the rape rhizosphere soil and a control soil.

Hinsinger and Gilkes (1997), found that rape and ryegrass (*Lolium rigidum* L.) had the greatest ability to solubilize phosphate rock compared to 3 other plant species grown in an acidic alumina sand media. However, the mechanisms of phosphate rock dissolution differed between rape and ryegrass. Rape took up the largest amount of Ca^{2+} of any species, thus promoting the dissolution of phosphate rock via calcium depletion of

the soil solution. Ryegrass, however, took up a very low amount of Ca^{2+}, and the authors suggested that it was through exudates of H^+ that ryegrass promoted dissolution of the phosphate rock. Rhizosphere pH of the rape increased from a pH of 4 to 7, 1.5 pH units more than would be expected from the production of OH^- due to phosphate rock dissolution. The authors suggested that the additional alkalinity was due to an excess of OH^- or HCO_3^- exuded by rape roots in order to promote P desorption from the alumina media. The rhizosphere pH of ryegrass and the other species tested did not differ from the initial starting point of 4.0. Marschner et al. (2007) also found that several *Brassica* species increased the rhizosphere pH of a P deficient soil from pH 4.8 to between pH 5.3 and 6.2. Such a change in pH could increase the solubility of Fe and Al phosphate 5 to 10 times. Wheat, used in the same study, slightly decreased the rhizosphere pH to 4.7 and did not acquire as much P from the soil as the *Brassicas* did.

Root exudation of organic acids is another mechanism through which *Brassicas* can increase the solubility and uptake of P. Organic acids and their dissociated anions, such as malate, citrate, and oxalate can precipitate with Ca^{2+} in soil solution, thus favoring the dissolution of calcium phosphate rocks. Organic anions can also chelate Fe^{3+} and Al^{3+}, preventing the formation of Fe and Al phosphate precipitates which are sparingly soluble. Finally, organic anions can occupy sorption sites on Fe and Al oxides, reducing the P sorption capacity of soil and releasing sorbed P via ligand exchange.

Hoffland et al. (1989) found that P deficient rape plants acidified their rhizosphere, and that this acidification was not due to a cation-anion uptake imbalance, but due to the exudation of citric and malic acids. Zhang et al. (1997) also found that P deficient radish (*Raphanus sativus* L.) and rape plants exuded 40 times the quantity of

organic acids under P deficient conditions compared to P sufficient conditions. Under P deficient conditions, radish exuded mainly tartaric acid (72%), followed by malic and succinic acids. Rape exuded mainly malic acid (80%), followed by citric and succinic acids. To test the abilities of each species to acquire P from different sources of sparingly soluble P, rape and radish were grown in sand culture with either $AlPO_4$ or $Ca_3(PO_4)_2$ and compared to control plants grown with a nutrient solution supplied with P as KH_2PO_4. Radish was able to acquire as much P from $AlPO_4$ as it did from the control, but only half as much P from $Ca_3(PO_4)_2$ as it did from the control. Rape, conversely, acquired more P from $Ca_3(PO_4)_2$, though only half as much as it acquired from the control, and it only acquired one quarter as much P from $AlPO_4$ as it did from the control. The authors suggested that rape and radish evolved the ability to exude different types of organic acids in accordance with the dominant forms of P that are found in the soils in the regions of China where each crop is grown. Radish, which in China is grown mostly in acidic soils, can chelate Fe^{3+} and Al^{3+} with exudates of tartaric acid, while rape, which in China is mostly grown in calcareous soils, may acidify the rhizosphere or precipitate Ca^{2+} with malic and citric acids, thus promoting dissolution of Ca phosphates.

In a survey of five mustard (*Brassica juncea* L.) varieties, Shahbaz et al. (2006) found a wide range of phosphorus utilization efficiencies between the varieties. Those varieties that were the most P-efficient were able to acidify a nutrient solution containing a Ca phosphate rock by 2 pH units and had greater rates of organic acid exudation than the less efficient varieties. All varieties of mustard increased their organic acid exudation in response to P deprivation, with the efficient varieties having the greatest increase. Surveying species for P-efficient varieties is an important task that can provide

information for plant breeders who are seeking to develop varieties that can grow well in P-deficient soils.

Rotations and intercrops of *Brassica* species have shown little effect in improving availability of P. When *Brassicas* are grown as a cash crop or as an intercrop, the soil P mobilized by the *Brassica* crop is removed at harvest or sequestered in plant tissue, rather than being cycled back into the soil as would be the case with a green manure crop.

Weil (2000) studied the interaction between phosphate rock application and crop rotation on corn yields. Previous crops of bean (*Phaseolus vulgaris* L.), pigeon pea, and corn increased the dry weight and P uptake of the following corn crop, while a previous crop of cabbage did not. The lack of positive effect of cabbage may be partially due to a decrease in mycorrhizal colonization following the cabbage crop compared to the other crops. The study did find that cabbage yield responded positively to amendment with an unreactive phosphate rock while the other crops tested did not. This was most likely due to the unique P acquisition strategies of *Brassica* crops and the findings suggested a means by which the unreactive phosphate rock could be used beneficially in the region. In another study, an intercrop of canola and wheat did not improve P uptake or yields of wheat in P deficient soils (Wang et al., 2007).

Brassicas used as a cover crop or green manure may be more effective than *Brassica* cash crops in improving subsequent P availability, but little work has been done on this topic. *Brassica* cover crops could be used to cycle soil P from forms that are relatively unavailable to organic P, which would then be mineralized to inorganic P following termination of the cover crop. Cavigelli and Thien (2003) found that P uptake of a sorghum (*Sorghum bicolor* L.) crop was positively correlated to the P uptake of a

previous perennial forage green manure crop. In low P soil, *Brassica* species may prove advantageous as green manure crops due to their ability to accumulate greater levels of tissue P.

There have been several reports of increased soil test P following *Brassica* cover crops. A cover crop of forage radish (*Raphanus sativus* L. var. *longipinnatus*) increased soil test P 10% at the 0 to 15 cm depth compared to 3 other *Brassica* cover crops and a sorghum-sudan grass (*Sorghum bicolor* L. *X S. sudanese* L.) cover crop (Wang et al., 2008). The forage radish increased marketable yield of the following onion crop, though it is unclear whether the onion crop was responding to the increased soil test P level. In a study reported by Grove et al. (2007), soil test P increased in the 0 to 45cm depth following three years of forage radish cover crops compared to rape, rye, and no cover crop treatments. The use of *Brassica* cover crops to increase plant available P needs to be studied further to determine their efficacy.

Conclusions

Cover crops are integral components of sustainable agricultural systems and provide many benefits for the environment and for farm management. There is significant potential to use cover crops to improve P availability to subsequent crops. One mechanism to achieve this is by using cover crops which host AMF and will maintain an active hyphal network through the winter and into the spring. The subsequent crop will benefit from rapid mycorrhizal colonization and an extensive hyphal network which can effectively supply P to the emerging root system. In management systems which require tillage in spring, the use of AMF host cover crops will be especially important to maintain the colonization potential of AMF.

Although *Brassicas* are AMF non-hosts and release ITCs upon decomposition, when used in typical cropping systems and natural soil, *Brassicas* appear to have no effective toxicity towards AMF. The concentration of ITCs released by *Brassicas* is generally below levels that are toxic to soil organisms and the ITCs that are released rapidly degrade in the soil. The negative effect that *Brassicas* have on AMF populations compared to host crops is more likely due to the natural atrophying of the AMF population which occurs during an extended period without a host crop.

Substantially greater concentrations of ITCs can be released by *Brassicas* when specific biofumigation practices such as macerating tissue, saturating the soil with water, and incorporating plant tissue through tillage are employed. This combination of management practices might prove more harmful to AMF than other typical cropping systems which include *Brassicas* in the rotation, but little research has been done on this topic. On high-input farms with optimum levels of soil phosphorus, negative effects of biofumigation practices on AMF may have little effect on subsequent yields. But biofumigation is also being promoted as a sustainable practice for low-input farms where phosphorus is often a limiting nutrient. Under such conditions, negative effects on AMF are more likely to translate into reduced crop yields, and the impact of biofumigation practices on AMF should be addressed.

Cover crops and green manures can also improve P availability by reducing the P sorption capacity of soil colloids. However, little work has been done on the use of *Brassica* green manure crops. The idea of using P mobilizing crops such as *Brassicas* to extract recalcitrant forms of P from soil and cycle them into more available forms of P has merit and should be investigated.

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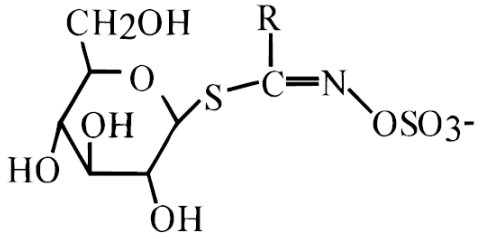
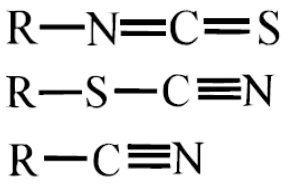
Glucosinolate Structure



Glucosinolate Hydrolysis Products



Common R Groups

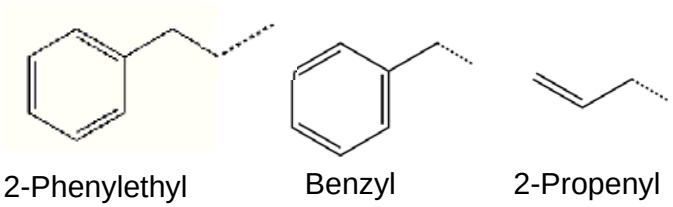


Figure 2.1. Structure of glucosinolates and their hydrolysis products. Figure compiled from Gardiner et al. (1999) and Halkier and Gershenzon (2006).

Chapter 3. Forage Radish and Cereal Rye Cover Crop Effects on Mycorrhizal Colonization of Corn Roots

Abstract

Forage radish (*Raphanus sativus* L. var. *longipinnatus*) is being used by increasing numbers of farmers as a winter cover crop in the Mid-Atlantic USA. It is a non-host to arbuscular mycorrhizal fungi (AMF) and releases anti-fungal isothiocyanates (ITCs) upon decomposition in the winter. Field experiments were conducted to determine the effect of forage radish and cereal rye (*Secale cereale* L.*)* cover crops on arbuscular mycorrhizal colonization of and P acquisition by a subsequent corn (*Zea mays* L.) silage crop. Cover crop treatments included forage radish, rye, a mix of forage radish and rye, and no cover crop. Mycorrhizal colonization of corn roots at the V4 stage following forage radish cover crops was not significantly different from that in the no cover crop treatment. In 3 out of 6 site-years, a rye cover crop increased AMF colonization of V4 stage corn roots compared to no cover crop. Across sites, AMF colonization of corn roots at the V4 stage was positively correlated with V4 and V8 stage corn shoot P concentration in both years of the study. These findings suggest that forage radish cover crops do not have a negative effect on AMF colonization of subsequent crops.

Key words: arbuscular mycorrhizal fungi, forage radish, cereal rye, phosphorus, isothiocyanate

Introduction

Arbuscular mycorrhizal fungi (AMF) form a symbiotic relationship with the roots of most agricultural crops and aid the roots of those crops in acquiring soil phosphorus (P) (Brundrett, 2004). The majority of AMF species are obligate symbionts, meaning they cannot survive over extended time periods in the absence of a host plant to provide them an energy source (Brundrett, 2002).

Numerous field studies have demonstrated the benefits of the mycorrhizal association to agricultural crops. Increased levels of root colonization and AMF hyphal density in soil at early growth stages (<V6 stage) can increase P uptake and yield in corn (*Zea mays L.*) when the soil is P deficient (Boswell et al., 1998; Deguchi et al., 2007; Gavito and Miller, 1998a; Kabir and Koide, 2002). In soil with adequate plant available P, increased mycorrhizal colonization has generally not translated into increased yields (Galvez et al., 2001; McGonigle and Miller, 1993; Sorensen et al., 2005).

Forage radish (*Raphanus sativus* L. var. *longipinnatus*) is being used in many parts of the world as a winter cover crop to alleviate soil compaction, reduce nitrate leaching, suppress weeds and control erosion (Weil and Kremen, 2007). In the Mid-Atlantic USA, forage radish is being used by an increasing number of dairy farmers as a cover crop between corn silage crops. Forage radish is a member of the *Brassica* family, one of the few plant families whose members do not host AMF (Ocampo et al., 1980; Vierheilig et al., 2000). Plants in the *Brassica* family also contain glucosinolates in their tissue, which can be hydrolyzed by the enzyme myrosinase to form isothiocyanates (ITCs), chemicals with anti-fungal properties (Schreiner and Koide, 1993a; Schreiner and Koide, 1993b; Vierheilig and Ocampo, 1990a; Vierheilig and Ocampo, 1990b; Vierheilig et al., 2000). However, isothiocyanates rapidly degrade in soil due to microbial

consumption and reactions with organic matter (Gardiner et al., 1999; Morra and Kirkegaard, 2002; Rumberger and Marschner, 2003).

Exposing AMF spores to *Brassica* root extracts in vitro reduced germination of the spores exposed to soluble ITCs and completely inhibited germination of spores exposed to volatile ITCs after 7 days (Vierheilig and Ocampo, 1990a; Vierheilig and Ocampo, 1990b). However, Schreiner and Koide (1993a; 1993b) observed that living *Brassica* roots in sterilized soil, or *Brassica* root extracts in fresh soil, reduced germination of AMF spores after 5 and 7 days, but after 12 or 14 days, germination had returned to control levels, suggesting that ITCs may only be fungistatic in their effect on AMF spores.

The literature is unclear as to whether the release of ITCs by *Brassicas* under field conditions is toxic to AMF. Some studies have shown that compared to a mycorrhizal host crop, *Brassica* crops reduce colonization of the subsequent crop during its early growth stages (Gavito and Miller, 1998b; Sorensen et al., 2005). Several studies, however, have shown that *Brassica* crops do not result in any lesser colonization of subsequent crops than a bare-fallow season does (Black and Tinker, 1979; Ocampo and Hayman, 1981; Ryan and Angus, 2003). One study found a reduction in AMF inoculum potential and colonization following a *Brassica* pre-crop as compared to a bare-fallow treatment, but the study was conducted in a potted mixture of sand and vermiculite, which may not degrade ITCs as effectively as a natural soil (Fontenla et al., 1999).

We found no studies that investigated the effect of a forage radish cover crop on the AMF colonization of the next crop. Forage radish exhibits several unique characteristics; some of which may increase negative effects on AMF and others which

may decrease negative effects on AMF. First, forage radish is grown as a winter cover crop between two successive summer crops rather than as a summer crop itself. As such, the duration between the mycorrhizal host crops grown prior to and following a forage radish cover crop is much shorter (~6 months) than the duration between mycorrhizal host crops when a Brassica is grown as a summer cash crop (>1 year). The hyphal networks of AMF that are established during the growth of a host crop can retain their colonization potential in undisturbed soil from fall to spring but the colonization potential decreases as the length of time without a host crop increases (Kabir et al., 1999; McGonigle and Miller, 1999).

The second difference between the potential effect of forage radish cover crops and other *Brassica* crops on AMF is the timing of the release of ITCs. When planted in fall as a cover crop, forage radish is killed at a vegetative growth stage when winter temperatures fall below -4 ^{o}C. Following its death, glucosinolates in the forage radish tissue are converted into ITCs and are released into the soil and atmosphere. Other *Brassica* crops, such as canola (*Brassica napus* L.), often aren't killed until after reproductive maturity is reached. Tissue concentrations of glucosinolates, the precursor compounds to ITCs, are at their highest during vegetative growth stages, decline after flowering, and are absent by senescence (Kirkegaard et al., 2000). On the other hand, ITC toxicity to soil organisms is reduced at low temperatures (Matthiessen and Shackleton, 2005), which are the case when forage radish cover crops winter-kill and presumably release ITCs into the soil.

The purpose of this study is to determine if forage radish cover crops have a negative effect on the AMF colonization and P acquisition of a subsequent corn crop.

The study also aims to compare the effects of a forage radish cover crop with the effects of a cereal rye cover crop. Rye, an AMF host, is a frequently used cover crop in the Mid-Atlantic USA, and is known to increase mycorrhizal colonization of the subsequent crop (Kabir and Koide, 2002). Finally, this study will test whether a mixed cover crop of forage radish and rye will increase AMF colonization of subsequent crops to the same extent as a pure stand of rye.

Materials and Methods

Experimental Design

Experiments were conducted at three sites: the University of Maryland Central Maryland Research and Education Center (CMREC), the USDA Beltsville Agricultural Research Center North Farm (BARC-NF), and the USDA Beltsville Agricultural Research Center South Farm (BARC-SF). The experiments lasted two complete years at each site, starting in August 2006 and ending in August 2008. Location and soil properties of the individual sites are listed in Table 3.1.

At all sites a randomized complete block experimental design with four replicates was used. There were three cover crop treatments common to all sites: forage radish, cereal rye (*Secale cereale* L. cv. 'Wheeler'), and no cover crop. At CMREC, an additional cover crop treatment was included: a rye/radish mixture planted in an arrangement of two rows of rye alternating with two rows of radish on 16 cm row spacing. The no cover crop treatment was maintained weed free with herbicides. At BARC-SF plots were 3 m wide by 15 m long, at CMREC plots were 6 m wide by 12 m long, and at BARC-NF plots were 3 m wide by 9 m long.

Site-specific Management Operations

CMREC. Prior to the start of the experiment, a rotation of corn, followed by winter wheat (*Triticum aestivum* L.), and double crop soybeans was grown from 2005 to 2006. The soybean crop was mowed at a vegetative stage in early August 2006 and left to decompose as a source of nitrogen to promote cover crop growth on the sandy soil at this site. When the soybeans were mowed, the above ground dry matter contained 56 kg N ha^{-1} with a C/N ratio of 13. The field had been managed using no-till practices since the fall of 2003 when it was last chisel plowed. Cover crops were planted on 12 September 2006 using a no-till drill with 16 cm row spacing. Rye was seeded at a rate of 135 kg ha^{-1} and forage radish was planted at a rate of 14 kg ha^{-1}. Because the rye/radish mixture was planted in an arrangement of two rows of rye alternating with two rows of radish, the seeding rate for each cover crop was effectively half of the rate used for seeding a pure stand. The forage radish cover crop was killed naturally when temperatures dropped below -4 ^{o}C in January and the rye cover crop was killed when all plots in the experiment were sprayed with paraquat dichloride (0.68 L ha^{-1} a.i.) and 2,4-D (2,4-dichlorophenoxyacetic acid) (1.05 L ha^{-1} a.i.) on 10 April 2007.

Corn (Pioneer 38B84, glyphosate tolerant) was planted at a rate of 74,000 seeds ha^{-1} with 75 cm row spacing on 23 April 2007. At planting, a 30% UAN solution was applied at the soil surface in a band 5 cm to the side of the seed furrow at a rate of 22 kg N ha^{-1}. Glyphosate (1.85 L ha^{-1} a.i.) was sprayed on 7 May 2007 to control weeds. The corn was side dressed with 112 kg N ha^{-1} as 30% UAN solution knifed in to a depth of 10 cm between every second corn row on 6 June 2007. The corn was harvested as silage on 16 August 2007.

To prepare the field for the second year of cover crops, weeds were killed with glyphosate (1.85 L ha^{-1} a.i.) and 22 kg N ha^{-1} as 30% UAN solution was applied as a starter fertilizer for the cover crops to ensure adequate growth. Cover crops were planted on 28 August 2007 in the same manner as the year before. Weeds in the no cover crop plots were controlled with glyphosate (1.85 L ha^{-1} a.i.) on 18 September 2007. The forage radish cover crop was killed naturally when temperatures dropped below -4 ^{o}C in January and the rye cover crop was killed when all plots in the experiment were sprayed with paraquat dichloride (0.68 L ha^{-1} a.i.) and 2,4-D (1.05 L ha^{-1} a.i.) on 12 April 2008.

On 16 April 2008 corn was planted using the same practices as in 2007. At planting, a 30% UAN solution was applied at the soil surface in a band 5 cm to the side of the seed furrow at a rate of 22 kg N ha^{-1}. Glyphosate (1.85 L ha^{-1} a.i.), atrazine (0.42 L ha^{-1} a.i.), s-metolachlor (0.43 L ha^{-1} a.i.), and mesotrione (2-[4-(methylsulfonyl)-2-nitrobenzoyl]$^{-1}$,3-cyclohexanedione) (0.05 L ha^{-1} a.i.) were applied on 16 May 2008 to control weeds. The corn was side dressed with 112 kg N ha^{-1} as 30% UAN solution knifed in to a depth of 10 cm between every second row on 6 June 2007. The corn was harvested as silage on 12 August 2008.

BARC-NF. Prior to the start of the experiment, potatoes (*Solanum tuberosum* L.) and green beans (*Phaseolus vulgaris* L.) were grown in 2005 and 2006 respectively. A cover crop of rye was established in the fall of 2005. The field had a long history of conventional tillage practices. Prior to the start of the experiment the field was chisel plowed in October 2005 and moldboard plowed in June 2006 and August 2006. The field was disked following each of these plowings. Once the experiment started, however, the field was managed using no-till practices to minimize soil disturbance.

Cover crops were planted on 31 August 2006 using a no-till drill with 16 cm row spacing. Rye was seeded at a rate of 135 kg ha^{-1} and forage radish was planted at a rate of 14 kg ha^{-1}. The forage radish cover crop was killed naturally when temperatures dropped below -4 ^{o}C in January and the rye cover crop was killed when all plots in the experiment were sprayed with paraquat dichloride (0.68 L ha^{-1} a.i.) on 11 April 2007.

Corn (Pioneer 38B84, glyphosate tolerant) was planted at a rate of 74,000 seeds ha^{-1} with 75 cm row spacing on 24 April 2007. Granular ammonium nitrate was applied in a band 5 cm to the side of the seed furrow and 5 cm deep at a rate of 22 kg N ha^{-1}. Glyphosate (1.85 L ha^{-1} a.i.) was sprayed on 9 May 2007 to control weeds. The corn was side dressed with 112 kg N ha^{-1} as 30% UAN solution dribbled on the soil surface between rows on 20 June 2007. The corn was harvested as silage on 16 August 2007.

To prepare the field for the second year of cover crops, weeds were killed with glyphosate (1.85 L ha^{-1} a.i.) and 22 kg N ha^{-1} as 30% UAN solution was dribbled on the soil surface in bands as a starter fertilizer for the cover crops to ensure adequate growth. Cover crops were planted on 27 August 2007 in the same manner as the year before. Weeds in the no cover crop plots were controlled with glyphosate (1.85 L ha^{-1} a.i.) on 3 October 2007. The forage radish cover crop was killed naturally when temperatures dropped below -4 ^{o}C in January and the rye cover crop was killed when all plots in the experiment were sprayed with glyphosate (1.85 L ha^{-1} a.i.) and 2,4-D (1.05 L ha^{-1} a.i.) on 16 April 2008.

On 7 May 2008, corn was planted using the same practices as in 2007. Granular ammonium nitrate was applied in a band 5 cm to the side of the seed furrow and 5 cm deep at a rate of 22 kg N ha^{-1}. Glyphosate (1.85 L ha^{-1} a.i.) was sprayed on 18 June 2008

to control weeds. The corn was side dressed with 112 kg N ha^{-1} as 30% UAN solution dribbled on the soil surface between rows on 17 June 2008. The corn was harvested as silage on 12 August 2008.

BARC-SF. Prior to the start of the experiment, sweet corn and soybeans (*Glycine max* L.) were grown in 2004 and 2005, respectively. Following the harvest of soybeans in the fall of 2005, the field remained fallow until the cover crop experiment was planted in August 2006. The field had a long history of conventional tillage practices. In 2006, the field was moldboard plowed in May and disked in June and July prior to the start of the cover crop experiment in August. Once the experiment started, however, the field was managed using no-till practices to minimize soil disturbance. On 30 August 2006 fertilizers were broadcast applied at rates of 84 kg ha^{-1} of K as potassium chloride, 17 kg ha^{-1} of P as triple super phosphate, and 62 kg ha^{-1} of N as urea.

Cover crops were planted on 31 August 2006 using a no-till drill with 16 cm row spacing. Rye was seeded at a rate of 135 kg ha^{-1} and forage radish was planted at a rate of 14 kg ha^{-1}. The forage radish cover crop was killed naturally when temperatures dropped below -4 ^{o}C in January and the rye cover crop was killed when all plots in the experiment were sprayed with glyphosate (N-(phosphonomethyl)glycine) applied at a rate of 1.85 L ha^{-1} active ingredient (a.i.) on 10 April 2007.

Corn (Pioneer 38B84, glyphosate tolerant) was planted at a rate of 74,000 seeds ha^{-1} with 75 cm row spacing on 24 April 2007. Granular ammonium nitrate was applied in a band 5 cm to the side of the seed furrow and 5 cm deep at a rate of 22 kg N ha^{-1}. The corn was side dressed with 112 kg N ha^{-1} as 30% urea ammonium nitrate (UAN) solution dribbled on the soil surface between rows on 07 June 2007. Glyphosate (1.85 L ha^{-1} a.i.)

was sprayed on 07 June 2007 to control weeds. The corn was harvested as silage on 16 August 2007.

To prepare the field for the second year of cover crops, weeds were killed with glyphosate (1.85 L ha^{-1} a.i.) and 22 kg N ha^{-1} as 30% UAN solution was dribbled on the soil surface in bands as a starter fertilizer for the cover crops to ensure adequate growth. Cover crops were planted on 27 August 2007 in the same manner as the year before. Weeds in the no cover crop plots were controlled with glyphosate (1.85 L ha^{-1} a.i.) on 4 October 2007 and paraquat dichloride (1,1'-dimethyl-4,4'-bipyridinium dichloride) (0.68 L ha^{-1} a.i.) on 05 November 2007. The forage radish cover crop was killed naturally when temperatures dropped below -4 ^{o}C in January and the rye cover crop was killed when all plots in the experiment were sprayed with glyphosate (1.85 L ha^{-1} a.i.) on 16 April 2008.

On 7 May 2008 corn was planted using the same practices as in 2007. Granular ammonium nitrate was applied in a band 5 cm to the side of the seed furrow and 5 cm deep at a rate of 22 kg N ha^{-1}. The corn was side dressed with 112 kg N ha^{-1} as 30% UAN solution dribbled on the soil surface between rows on 10 June 2008. Glyphosate (2.77 L ha^{-1} a.i.), atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) (1.49 L ha^{-1} a.i.), and s-metolachlor (acetamide, 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy^{-1}-methylethyl]-,(S)) (1.61 L ha^{-1} a.i.) were sprayed on 10 June 2008 to control weeds. The corn was harvested as silage on 12 August 2008.

Cover Crop Dry Matter Sampling

The shoots of the forage radish cover crop were sampled near the time of maximum dry matter accumulation in the late fall. The shoots of the rye cover crop were

sampled in the spring, prior to being killed by herbicides. Exact dates of cover crop sampling are listed in Table 3.2. Cover crop samples were obtained by removing plant parts from two 0.25 m^2 quadrats in each plot. Forage radish roots and shoots were separated in the field. All plant parts were washed to remove attached soil prior to drying in a forced draft oven at 60 ºC for a minimum of 7 days. Samples were weighed then ground and sieved to <2 mm particle size and stored in sealed polyethylene vials.

Corn Root and Shoot Sampling

Corn roots and shoots were sampled for analysis of mycorrhizal colonization, dry matter accumulation, and tissue phosphorus content at the V4 (4th leaf), V8 (8th leaf) and R1 (silking) growth stages. Exact dates of sampling are listed in Table 3.2. The field plots at USDA-NF and USDA-SF contained 4 corn rows and plant samples were removed from the center 2 rows. At CMREC field plots contained 8 corn rows and plant samples were taken from the third and sixth row of each plot, except in the rye/radish mixed plots. In these plots, two corn rows which were positioned between a radish and a rye cover crop row were selected for sampling. At each sampling date, 4 consecutive plants were removed from each sampling row for a total of 8 plants from each plot per sampling date. The length of row which the 8 plants occupied was recorded in order to calculate dry matter production and nutrient uptake on a per hectare basis. In each sampling row, the V4 samples were taken starting from a randomly selected plant approximately 1 m inside the plot border. The V8 samples were taken from the same row as the V4 samples. Approximately 1 m of row was skipped between where the V4 samples had been taken from and where the first plant of the V8 sample was randomly selected. The R1 samples were selected in a similar manner as the V8 samples. The side

of the plot from which the corn sampling at the V4 stage began was randomly selected by block in the first year of sampling. In the second year of sampling, the side of the plot from which the sampling began was opposite to the side started from in the first year in order to eliminate any interference due to overlapping of sampling locations from year to year.

Corn shoots were cut at the soil surface and the 8 shoots per plot were combined into a composite sample. Each sample was rinsed in distilled water prior to drying in a forced draft oven at 60 ºC for a minimum of 7 days. Samples were weighed then ground and sieved through a 2 mm screen and stored in sealed polyethylene vials.

Following removal of shoots, at the V4 and V8 stages, roots were sampled from the same corn plants by removing a soil core centered over the cut base of each plant. In 2007, cores measured 10 cm in diameter and 10 cm in depth. In 2008, cores measured 7 cm in diameter and 10 cm in depth. The soil cores containing roots from the 8 plants were pooled into a composite sample and the majority of soil was washed from the roots in the field. Root samples were stored at 4 ºC for a maximum of 24 hours before being washed to remove all soil particles. Root samples were then immediately dried in a forced draft oven at 60 ºC and stored until analysis for mycorrhizal colonization.

Silage Yield Sampling

Samples to determine silage yield were collected from the same corn rows which were sampled in the earlier growth stages. All plants in two 3 m sections of row from each plot were cut 5 cm above the soil and weighed fresh in the field. Three of the sampled whole plants were randomly selected from each plot to be dried in a forced draft

oven at 60 ºC for a minimum of 7 days to determine the moisture content, which was used to calculate dry matter weight of the silage corn.

Plant Tissue Phosphorus Analysis

Phosphorus in plant tissue was determined by ashing 0.4 g of each tissue sample in a muffle furnace at 550 ºC for five hours. The ash was dissolved in 40 mL of 0.3 M HCl and filtered through Whatman No. 42 filter paper (Whatman International, Maidstone, UK). Phosphorus in the filtrate was measured colorimetrically using the vanadomolybdophosphoric acid method (Kuo, 1996) on a spectrophotometer set to 420 nm (DU720, Beckman-Coulter, Inc., Fullerton, CA).

Mycorrhizal Colonization Analysis

The dried corn root systems were separated into fine roots (< 1 mm diameter) and coarse roots with only the fine roots analyzed for mycorrhizal colonization. The fine roots were cut into 1 cm segments and a random sample of approximately 75 mg dry weight of roots were packed into histology cassettes for clearing and staining. Roots were cleared and stained using a modification of the procedure by Koske and Gemma (1989). Roots were cleared in 10% KOH (w/v) at room temperature for 16 hours and stained in 0.05% Trypan Blue (w/v) stain at room temperature for 6 hours. Stained roots were placed on an 8 cm x 8 cm Petri dish and viewed under a dissecting microscope (SMZ-2T, Nikon, Tokyo, Japan) at 60X magnification. Mycorrhizal colonization was assessed as a percentage of root length colonized using the grid-line intersect method (Giovannetti and Mosse, 1980) which also yielded an estimate of the total root length of the sample (Newman, 1966). Total mycorrhizal colonization was recorded, which included colonization by hyphal, arbuscular, and vesicular fungal structures.

Statistical Analysis

Data were analyzed by ANOVA in the Mixed procedure of SAS (SAS Institute, Cary, NC). To maximize the power of the analysis, data from all sites and years were pooled in the ANOVA for the cover crop experiment. However, because of the unbalanced treatments, with a mixed cover crop only at CMREC, comparisons between means were restricted to within sites. In the model, sites and blocks were considered random factors and cover crop and year were considered fixed factors. Year was treated in the experimental design as a split-plot factor within cover crop main plots. Data from each stage of corn growth were analyzed separately. When the ANOVA indicated a statistically significant treatment effect ($P<0.05$), mean comparisons were made using Fisher's Least Significant Difference test. Prior to analysis by ANOVA and making mean comparisons, measurements of proportional root length colonized by AMF were arcsin-square root transformed to meet assumptions of normality. Root colonization values reported in the text are back transformed. Correlations between variables were determined using Pearson's correlation co-efficient in the Corr procedure of SAS (SAS Institute, Cary, NC).

Results

Cover crop growth

Dry matter production of cover crops varied among sites and between years (Table 3.3). At CMREC in both years, cover crops produced less dry matter than at the other sites. For forage radish, this was due to a late planting date in 2006 and because of nitrogen deficiencies on this loamy sand soil in both years, which were identified based on visual observations of chlorosis of the oldest leaves. The low dry matter production of rye at CMREC may have also been related to nitrogen deficiencies, although chlorotic

leaves were not observed. The soil at CMREC is a coarser texture than at the other sites which may have caused residual nitrogen to leach beyond the rooting zone of the cover crops faster than at the other sites. Also, the soil at CMREC has less organic matter than the other sites, so is likely to supply less mineralized N during the growth of cover crops in the fall and spring. Finally, the mowed soybeans in 2006 may have provided insufficient N for cover crop growth. At BARC-NF and BARC-SF rye and forage radish growth was normal and achieved shoot dry matter production over 4,000 kg ha^{-1} in all years and exceeded 7,000 kg ha^{-1} of rye at BARC-NF in 2008 and BARC-SF in 2007.

Mycorrhizal Colonization

There was a cover crop effect on the percent of root length colonized by AMF at the V4 stage of corn growth (Table 3.4). There was also a significant Site X Year interaction on AMF colonization at both V4 and V8 growth stages. There was no cover crop effect at any site on AMF colonization of corn roots at the V8 growth stage (Table 3.4).

At CMREC, the rye cover crop caused greater V4 corn root colonization than any of the other cover crop treatments in both 2007 and 2008 (Table 3.5). There was no difference in V4 corn root colonization between the forage radish, mix, and no cover crop treatments at CMREC in either year. At BARC-NF, there were no differences in V4 corn root colonization between the cover crop treatments in 2007 but in 2008 the rye cover crop caused greater V4 corn root colonization than no cover crop. At BARC-SF in 2007, the rye cover crop caused greater V4 corn root colonization than the forage radish cover crop. At BARC-SF in 2008 there were no significant differences in V4 corn root colonization between the cover crop treatments. Corn root colonization at the V4 stage

never differed between the forage radish cover crop treatment and the no cover crop treatment in any site-year.

At the V4 stage, corn roots at all sites had greater levels of colonization in 2008 than they did in 2007, with BARC-SF showing the greatest increase (~ 3X) between years (Table 3.5). At the V8 stage, corn roots had greater levels of colonization in 2008 compared to 2007 at CMREC and BARC-SF, but at BARC-NF there was no colonization difference between years.

Corn Shoot P concentration

There were significant interactions between cover crop and site at the V4 stage and cover crop and year at the V8 stage. At the R1 (silk) stage, cover crop had a significant effect on corn shoot P concentration (Table 3.4). There was also a significant year by site interaction at all growth stages.

At CMREC, V4 corn shoots following a rye cover crop had a greater P concentration than those following a radish, mixed, or no cover crop in both 2007 and 2008 (Table 3.5). At CMREC in 2008, V4 corn shoots following forage radish had a lower shoot P concentration than those following all other treatments. At BARC-NF and BARC-SF, there were no significant differences between cover crop treatments in V4 corn shoot P concentration in either year.

In 2007, there were no significant differences between cover crop treatments in V8 corn shoot P concentrations at any of the sites (Table 3.5). In 2008, V8 corn shoots following a rye cover crop had a greater P concentration than those following any other treatment at both CMREC and BARC-NF. At CMREC, V8 corn shoots following a radish cover crop had a lower P concentration than those following a mixed cover crop.

At BARC-SF, there were no significant differences between cover crop treatments for V8 corn shoot P concentration.

At CMREC, R1 stage corn shoots had a greater P concentration following a rye cover crop than all other cover crop treatments in 2008, and a greater P concentration than no cover and forage radish treatments in 2007. Across all sites and corn growth stages, corn shoot P concentration was greater in 2008 than in 2007. The magnitude of the difference was greater at CMREC and BARC-SF than it was as BARC-NF.

Corn Dry Matter

There were significant interactions between cover crop, site, and year for corn dry matter production at the V4 and R1 growth stages and at silage harvest (Table 3.4). At the V8 growth stage, there was a significant interaction between cover crop and site for corn dry matter production. At CMREC in 2007, forage radish increased corn dry matter compared to both no cover crop and the mixed cover crop at the V8 stage and compared to no cover crop at the R1 stage (Table 3.6). There were no other significant differences in corn growth in 2007. In 2008 at CMREC, all cover crop treatments increased corn dry matter compared to no cover crop at the V8 and R1 stages. At the V4 stage and at silage harvest, forage radish resulted in greater corn dry matter compared to no cover crop.

At BARC-NF in 2007, both forage radish and rye increased corn dry matter at the V4, V8, and R1 stages compared to no cover crop (Table 3.6). Both forage radish and rye also increased silage yield compared to no cover crop. In 2008, compared to no cover crop, radish increased corn dry matter at the V4 stage and rye increased corn dry matter at the V8 stage. Cover crops did not affect silage yields in 2008 at BARC-NF, however.

At BARC-SF in 2007, the rye cover crop decreased corn dry matter at the V4 stage, but increased silage yield compared to no cover crop (Table 3.6). In 2008 at BARC-SF, the rye cover crop decreased corn dry matter at all growth stages including silage harvest compared to both forage radish and no cover crop. In both years at BARC-SF, forage radish and no cover crop treatments resulted in equivalent corn growth at all stages.

Corn Shoot P uptake

There were significant interactions between cover crop, site, and year for corn P uptake at the V4, V8, and R1 growth stages (Table 3.4). At CMREC in 2007, all cover crop treatments increased corn shoot P uptake compared to no cover crop at both the V4 and R1 stages (Table 3.5). At the V8 stage, only the radish cover crop increased corn shoot P uptake compared to no cover crop. In 2008 at CMREC, rye caused greater corn shoot P uptake compared to no cover crop at all corn growth stages and the mixed and radish cover crops caused greater corn P uptake compared to no cover crop at the V8 and R1 stages.

At BARC-NF in 2007, both rye and radish cover crops caused greater corn shoot P uptake compared to no cover crop at all growth stages and rye was greater than radish at the V4 stage (Table 3.5). In 2008 at BARC-NF, there were no significant differences between cover crop treatments at the V4 stage, but at the V8 stage corn P uptake was greater after a rye cover crop than it was after either a radish or no cover crop.

At BARC-SF in 2007, the rye cover crop resulted in less P uptake by corn shoots at the V4 stage than after no cover crop (Table 3.5). At the V8 and R1 growth stages, however, there was no significant difference between the cover crop treatments. In 2008

at BARC-SF, rye resulted in less P uptake by corn shoots compared to the other treatments at the V4 and V8 stage. At the R1 stage, the rye cover crop resulted in less P uptake by corn shoots compared to the forage radish cover crop, but was not significantly different than no cover crop.

Discussion

Mycorrhizal Colonization

Forage radish cover crops never caused decreased levels of AMF colonization of the subsequent corn crop compared to growing no cover crop at all. This finding suggests that ITCs released by forage radish had no toxic effect on AMF. There may be several reasons for the apparent lack of toxicity.

One reason could be that some portions of the AMF mycelium may never have contacted ITCs released by forage radish. Forage radish shoots, which make up most of the dry matter of the plant, decompose on the soil surface. Glucosinolates in *Brassica* shoots tend to produce volatile ITCs (Gardiner et al., 1999; Kirkegaard and Sarwar, 1998; Sarwar et al., 1998; Vierheilig et al., 2000), so a large portion of the ITCs released by forage radish probably diffused into the air rather than the soil. Because the soil was not tilled and plant parts were not evenly distributed throughout the soil, ITCs may have only been released into the zones of soil surrounding the forage radish roots, leaving other areas of soil unaffected.

The ITCs released into the soil by decomposing roots may never have been within the toxic range or may have been rapidly consumed by microbial degradation and reactions with soil organic matter. Other studies have reported that unless specific biofumigation procedures were followed, which included mechanical maceration of plant

parts and thorough mixing into the soil, toxic concentrations of ITCs were not achieved (Gardiner et al., 1999; Gimsing and Kirkegaard, 2006; Morra and Kirkegaard, 2002). Finally, the cold soil temperatures during the period of time when ITCs were released after the radish was killed by freezing may have decreased the toxicity of ITCs to AMF (Matthiessen and Shackleton, 2005).

This finding that forage radish did not reduce AMF colonization of the subsequent crop compared to no cover crop is consistent with several studies of *Brassica* crops that found no effect when compared to a fallow treatment (Black and Tinker, 1979; Ocampo and Hayman, 1981; Pellerin et al., 2007; Ryan and Angus, 2003). Some studies have concluded that a rotation of *Brassica* crops reduces subsequent AMF colonization, but these have been in comparison to a rotation of a mycorrhizal host crop rather than a fallow year (Gavito and Miller, 1998b; Sorensen et al., 2005).

At CMREC, a rye cover crop resulted in increased AMF colonization of the next corn crop compared to the other treatments. This is consistent with the findings of many others that cover crops which host AMF result in increased colonization of the subsequent crop (Boswell et al., 1998; Deguchi et al., 2007; Kabir and Koide, 2000; Kabir and Koide, 2002; Karasawa et al., 2001; Sorensen et al., 2005). At BARC-NF, mycorrhizal colonization was greater following rye only when compared to no cover crop. At BARC-SF there were no differences in AMF colonization of corn roots due to cover crops.

Several factors that distinguish the BARC-SF and BARC-NF fields from the field at CMREC may have limited the ability of rye to increase mycorrhizal colonization in the BARC fields. The first factor is that BARC-SF and BARC-NF were both subjected to

frequent tillage events in the years prior to the beginning of this study. By destroying the mycelium of AMF, tillage reduces the ability of AMF to colonize the roots of subsequent crops (Evans and Miller, 1990). Garcia et al. (2007) found that one tillage event reduced mycorrhizal colonization of subsequent crops for over 2 years with no indication of recovery during the time frame of the experiment. The second factor is that soil test P concentrations at BARC-SF and BARC-NF were greater than at CMREC. High soil P concentrations tend to reduce mycorrhizal colonization (Kabir and Koide, 2002; Koide and Li, 1990; Menge et al., 1978; Nadian et al., 1996) because the symbiotic association with AMF is not worth the energy cost to the plant when P is easily available in the soil. The greater soil P concentrations at the two BARC sites may have been a limiting factor to AMF colonization regardless of cover crop treatment.

An unexpected finding of our study is that at the CMREC site, a mixed cover crop stand of rye and forage radish did not result in increased levels of AMF colonization of the next crop even though the pure stand of rye did. Several studies have documented that the presence of a living *Brassica* root does not hinder AMF from colonizing a host root (Giovannetti et al., 1994; Glenn et al., 1988; Ocampo et al., 1980). However, these studies did not investigate if killing the *Brassica*, which would release ITCs into the soil, would have a negative effect on the AMF symbiosis with the host plant. Although no reduction of subsequent AMF colonization potential was observed in the pure radish stand, the ITCs released during radish decomposition may have affected the ability of rye to host AMF in the mixed cover crop stand. Another possibility is that the phosphorus released by the decomposing forage radish tissues acted as a fertilizer for the rye and

reduced its need to maintain a symbiosis with AMF. Further research into this phenomenon is warranted.

Another interesting finding is that AMF colonization of corn roots was greater in the second year of the study than in the first except for the V8 stage at BARC-NF. The most likely explanation for the greater colonization in the second year is that the warmer and wetter than normal weather conditions in the spring of 2008 may have been more favorable for mycorrhizal colonization than the cooler and drier conditions in the spring of 2007 (Figure 3.1). The favorable weather in spring 2008 may have improved early corn growth, allowed the corn plants to allocate more resources to supporting AMF colonization, and may have stimulated more rapid AMF spore germination and hyphal growth.

Corn Growth

In 5 of the 6 site years, at least one of the cover crop treatments increased corn dry matter production at one or more of the growth stages measured. The most notable effect on corn growth was at BARC-NF in 2007, where rye and forage radish cover crop treatments both resulted in increased corn dry matter through the entire growing season. Other instances of increased yield following a cover crop occurred at CMREC in 2008 following forage radish and at BARC-SF in 2007 following rye. In 2007, which was a drier than average year (Figure 3.1), increased corn growth due to cover crops could have been a result of improved access to water. Rye cover crops can reduce evaporative losses of soil moisture because of the residue mulch they leave on the surface. Forage radish cover crops, which leave behind root channels deep into the subsoil, can increase the

number of roots of the subsequent crop which grow deep into the soil and can access subsoil moisture (Williams and Weil, 2004).

Williams and Weil (2004) found that a mixed cover crop of forage radish and rye improved soybean yields compared to either cover crop grown alone or no cover crop at all. They suggested that the mixed cover crop stand increased water availability in both the surface and subsurface soil layers at the same time because of the residue mulch created by rye and the deep root channels created by forage radish. In this study, we did not find that the mixed cover crop stand increased yields. We only tested a mixed cover crop stand at the CMREC site, and in 2007, poor cover crop growth at this site (Table 3.3) probably limited any benefit of the cover crop. In 2008, when cover crop growth at CMREC was improved, rainfall was above average for much of the summer, so water availability was unlikely to be a limiting factor to the corn growth.

At BARC-SF in 2008, the rye cover crop decreased corn dry matter throughout the entire season. Corn was planted in this field in a brief window of clear weather between two periods of heavy rain. While soil moisture conditions were optimal for corn planting in the no cover and forage radish plots, the soil was probably too wet under the rye mulch, resulting in poor performance of the no-till planter. The seeding date was followed by a period of cold rainy weather and corn seed germination in the rye plots was poor, leading to a low stand count (data not shown). Others have reported similar problems with no-till corn planting into rye residue in wet years (Duiker and Curran, 2005). Although individual plants in the rye plots grew larger than those plants in the forage radish and no cover plots (data not shown), this was not sufficient to overcome the effect of such a low plant density on dry matter production on a per hectare basis. In

2007 at BARC-SF, rye also decreased corn growth at the V4 stage compared to forage radish and no cover crop, although corn growth eventually caught up at later stages. Rye cover crop dry matter was greater at BARC-SF than the other sites in both years, and the heavy mulch left behind by the cover crop may have negatively affected the early growth of the corn by decreasing soil temperatures and increasing soil water content.

Relationship Between AMF colonization, corn shoot P, and corn growth

Colonization of corn roots by AMF at the V4 stage was positively correlated with corn shoot P concentrations at the V4 and V8 stage in both years when data across sites was combined (Table 3.7, Table 3.8, Figure 3.2). Across all sites, in 2008 AMF colonization at the V4 stage was positively correlated with corn dry matter production at the V4 and V8 stages and at silage harvest (Table 3.8, Figure 3.3). In 2007 however, AMF colonization at the V4 stage was not correlated with silage yields (Table 3.7, Figure 3.3). Other studies have found correlations between AMF colonization, shoot P concentrations and yield in P deficient soil (Boswell et al., 1998; Deguchi et al., 2007; Gavito and Miller, 1998a; Kabir and Koide, 2002). However, most studies conducted in soil with optimum levels of P have not found a correlation between AMF colonization and yield as was found in 2008 in this study (Galvez et al., 2001; Galvez et al., 1995; McGonigle and Miller, 1993; Sorensen et al., 2005).

Conclusions

This research found that a forage radish cover crop did not effect AMF colonization of a subsequent corn crop despite the presumed release of ITCs during its decomposition. The lack of a negative effect might be accounted for by the no-till management of the cover crops by which the residues were not incorporated into the soil

and therefore any ITCs released would not have thoroughly permeated the soil at toxic levels. However, the finding that a mixed stand of forage radish and rye did not increase AMF colonization of the subsequent crop while a pure stand of rye did, suggests that forage radish may have inhibited rye's ability to host and maintain AMF. Further research in this area is necessary. Finally, further research in P deficient soil is suggested, as the importance of AMF in aiding in P acquisition is greater in such a soil and the effect of an AMF non-host cover crop as compared to a host cover crop may also be greater.

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Table 3.1. Selected properties of experimental sites and soils.

	Site		
	CMREC	BARC-NF	BARC-SF
Location	Greenbelt, MD	Beltsville, MD	Beltsville, MD
Latitude	39° 00' 42" N	39° 00' 51" N	39° 02' 01" N
Longitude	76° 49' 54" W	76° 56' 31" W	76° 55' 53" W
Hectares	0.57	0.40	0.93
Slope (%)	4	4	0.5
Soil Series	Downer	Hammonton	Codorus
Soil Taxonomy	Coarse-loamy, siliceous, semiactive, mesic Typic Hapludult	Coarse-loamy, siliceous, semiactive, mesic Aquic Hapludult	Fine-loamy, mixed, active, mesic Fluvaquentic Dystrudept
Surface (0-10 cm) soil properties			
pH_w	5.9 (0.30)[a]	5.6 (0.08)	6.9 (0.13)
Organic Matter[b] (g $100g^{-1}$)	1.1 (0.14)	1.3 (0.18)	1.6 (0.05)
Mehlich 3 P (mg kg^{-1})	88 (5.31)	102 (5.98)	98 (1.72)
Sand[c] (g $100g^{-1}$)	78	54	50
Silt (g $100g^{-1}$)	17	42	38
Clay (g $100g^{-1}$)	5	4	12

[a]Where multiple samples were measured, standard error is listed in parentheses; (*n*=4)
[b]Soil Organic matter by loss on ignition
[c]Particle size analysis by the hydrometer method

Table 3.2. Schedule of sampling and planting dates.

	Site		
	CMREC	BARC-NF	BARC-SF
Cover Crop Planting	12 September 2006	31 August 2006	31 August 2006
Cover Crop Sampling	15 November 2006	29 November 2006	15 November 2006
	01 April 2007	07 April 2007	01 April 2007
Cover Crop Planting	28 August 2007	27 August 2007	27 August 2007
Cover Crop Sampling	09 November 2007	20 November 2007	09 November 2007
	07 March 2008	11 April 2008	07 March 2008
Corn Planting	23 April 2007	24 April 2007	24 April 2007
Corn Sampling			
V4 growth stage	15 May 2007	18 May 2007	15 May 2007
V8 growth stage	13 June 2007	25 June 2007	11 June 2007
R1 growth stage	09 July 2007	11 July 2007	06 July 2007
Silage Harvest	07 August 2007	14 August 2007	07 August 2007
Corn Planting	16 April 2008	07 May 2008	07 May 2008
Corn Sampling			
V4 growth stage	19 May 2008	06 June 2008	09 June 2008
V8 growth stage	10 June 2008	25 June 2008	20 June 2008
R1 growth stage	30 June 2008	n.d.[a]	14 July 2008
Silage Harvest	04 August 2008	13 August 2008	06 August 2008

[a]n.d. = Not determined

Table 3.3. Dry matter production of cover crops at each site year. Values indicate the means (SE); *n*=4.

Cover	Dry Matter
CMREC 2007	—kg ha^{-1}—
Radish[a]	1306 (260)
Rye[b]	1711 (260)
Radish/Rye Mixed	
Radish in Mix	771 (260)
Rye in Mix	869 (260)
Total Mixed	1640 (260)
BARC-NF 2007	
Radish	5583 (569)
Rye	4177 (569)
BARC-SF 2007	
Radish	4282 (404)
Rye	7345 (404)
CMREC 2008	
Radish	2629 (260)
Rye	1546 (260)
Radish/Rye Mixed	
Radish in Mix	1821 (272)
Rye in Mix	763 (272)
Total Mixed	2584 (272)
BARC-NF 2008	
Radish	4642 (569)
Rye	7062 (569)
BARC-SF 2008	
Radish	4026 (404)
Rye	4117 (404)

[a]Radish shoots were sampled in the late fall.
[b]Rye shoots were sampled in spring.

Table 3.4. Significance of treatment effects on AMF colonization of corn roots, corn shoot P concentration, corn shoot P uptake, and corn dry matter production at various growth stages. To maximize power, all site years were pooled into a single ANOVA. Values indicate the probability of a greater F-value (α=0.05).

		Root Colonization		Shoot P Concentration			Shoot P Uptake			Dry Matter Production			
Source of variation	DF[a]	V4	V8	V4	V8	Silk	V4	V8	Silk	V4	V8	Silk	Yield
Cover	21	<0.001	0.984	0.001	0.002	<0.001	0.083	0.006	<0.001	0.008	0.010	<0.001	0.086
Year	27	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.025	<0.001	<0.001	<0.001	0.167	<0.001
Cover x Year	27	0.239	0.891	0.187	0.002	0.452	0.028	0.132	0.705	0.005	0.126	0.705	0.043
Site	0	.	.	.	.	.	.	.	.	.	.	.	.
Cover x Site	21	0.088	0.716	0.003	0.099	0.628	0.004	<0.001	0.042	0.002	0.004	0.143	0.029
Year x Site	27	<0.001	<0.001	<0.001	0.006	0.016	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.005
Cover x Year x Site	27	0.673	0.809	0.262	0.180	0.818	0.019	0.006	0.014	0.019	0.060	0.046	0.002

[a]DF= error degrees of freedom

Table 3.5. Cover crop effects on AMF colonization of corn roots, corn shoot P concentration, and corn shoot P uptake at various growth stages. Within site and year, means followed by different letters are significantly different (F-protected LSD, P<0.05).

	Corn Root Colonization		Corn Shoot P Concentration			Corn Shoot P Uptake		
Cover	V4	V8	V4	V8	Silk	V4	V8	Silk
	%		g kg^{-1}			kg ha^{-1}		
CMREC 2007								
Mix	31a	37[a]	3.87a	4.49a	2.62ab	0.078bc	3.03ab	15.2b
No Cover	32a	37	3.75a	4.18a	2.45a	0.056a	2.89a	12.4a
Radish	33a	38	3.81a	4.04a	2.41a	0.069ab	4.18b	15.0b
Rye	46b	37	5.27b	4.54a	2.79b	0.095c	3.35ab	15.2b
BARC-NF 2007								
No Cover	14a	51	2.49a	2.85a	2.52a	0.021a	3.15a	6.81a
Radish	14a	56	1.84a	3.02a	2.43a	0.031b	5.55b	10.4b
Rye	18a	49	2.07a	3.35a	2.65a	0.042c	6.41b	11.2b
BARC-SF 2007								
No Cover	22ab	36	2.28a	3.66a	2.43a	0.042b	2.71a	12.6a
Radish	18a	32	1.82a	4.04a	2.43a	0.030ab	3.21a	14.5a
Rye	27b	33	1.79a	3.93a	2.70a	0.023a	2.58a	15.0a
CMREC 2008								
Mix	62a	61	6.43b	5.22b	3.21a	0.206ab	4.19bc	13.6bc
No Cover	61a	59	6.57b	5.01ab	3.18a	0.174a	1.95a	9.34a
Radish	66a	63	5.19a	4.47a	3.18a	0.177a	3.88b	12.9b
Rye	77b	61	8.67c	6.30c	3.64b	0.235b	4.77c	15.2c
BARC-NF 2008								
No Cover	32a	48	3.05a	3.20a	n.d.	0.054a	1.62a	n.d.[b]
Radish	40ab	45	2.76a	2.82a	n.d.	0.063a	1.73a	n.d.
Rye	44b	45	3.72a	4.10b	n.d.	0.080a	2.96b	n.d.
BARC-SF 2008								
No Cover	70a	54	5.45a	4.62a	3.00a	0.672b	4.32b	21.7ab
Radish	72a	53	5.62a	4.46a	3.00a	0.641b	3.81b	23.3b
Rye	72a	57	5.59a	4.67a	3.26a	0.209a	2.34a	18.6a

[a]Mean comparisons were not made for V8 corn root colonization because the ANOVA did not indicate a significant cover crop treatment effect.
[b]n.d. = Not determined

Table 3.6. Cover crop effects on corn dry matter production at various growth stages. Within site and year, means followed by different letters are significantly different (F-protected LSD, P<0.05).

Cover	Stage			
	V4	V8	Silk	Silage
	kg ha^{-1}			
CMREC 2007				
Mix	20.4a	683a	5817ab	9543a
No Cover	16.3a	715a	5094a	9763a
Radish	18.8a	1045b	6254b	10198a
Rye	18.7a	745ab	5483ab	9241a
BARC-NF 2007				
No Cover	9.0a	1123a	2708a	7225a
Radish	17.7b	1854b	4290b	9870b
Rye	20.2b	1895b	4233b	10959b
BARC-SF 2007				
No Cover	17.6b	749a	5205a	12157a
Radish	16.9ab	791a	5980a	12755ab
Rye	13.2a	654a	5602a	14798b
CMREC 2008				
Mix	32.4ab	807b	4249b	13911ab
No Cover	26.6a	400a	2949a	11825a
Radish	34.0b	868b	4090b	14721b
Rye	28.1ab	759b	4163b	14008ab
BARC-NF 2008				
No Cover	16.8a	502a	n.d.[a]	8548a
Radish	22.5b	617ab	n.d.	10164a
Rye	21.4ab	706b	n.d.	11213a
BARC-SF 2008				
No Cover	122b	937b	7128b	16046b
Radish	113b	848b	7789b	17020b
Rye	36.9a	501a	5698a	9759a

[a]n.d. = Not determined

Table 3.7. Correlations between corn shoot dry matter, corn shoot P concentration, and AMF root colonization in 2007. Significant Pearson correlation coefficients are given (P<0.01).

	DM V4	DM V8	DM Silk	DM Silage	P V4	P V8	P Silk	AMF V4	AMF V8
DM[a] V4	1	n/s[d]	0.50	n/s	n/s	n/s	n/s	n/s	n/s
DM V8		1	n/s	n/s	n/s	-0.55	n/s	-0.48	0.60
DM Silk			1	0.44	n/s	0.43	n/s	0.42	-0.53
DM Silage				1	n/s	n/s	n/s	n/s	n/s
P[b] V4					1	0.65	n/s	0.79	n/s
P V8						1	0.41	0.69	-0.43
P Silk							1	n/s	n/s
AMF[c] V4								1	-0.44
AMF V8									1

[a] DM = Corn shoot dry matter in kg ha^{-1} at each stage
[b] P = Corn shoot P concentration at each stage
[c]AMF = Percentage of corn root length colonized by AMF at each stage
[d]n/s= Not significant

Table 3.8. Correlations between corn shoot dry matter, corn shoot P concentration, and AMF root colonization in 2008. Significant Pearson correlation coefficients are given (P<0.01).

	DM V4	DM V8	DM Silk	DM Silage	P V4	P V8	P Silk	AMF V4	AMF V8
DM[a] V4	1	0.60	0.88	0.65	n/s	n/s	-0.48	0.44	n/s
DM V8		1	0.59	0.64	n/s	n/s	n/s	n/s	n/s
DM Silk			1	0.82	n/s	n/s	-0.48	n/s	-0.58
DM Silage				1	0.50	0.49	n/s	0.71	n/s
P[b] V4					1	0.88	0.63	0.81	0.65
P V8						1	0.85	0.70	0.54
P Silk							1	n/s	n/s
AMF[c] V4								1	0.55
AMF V8									1

[a] DM = Corn shoot dry matter in kg ha^{-1} at each stage
[b] P = Corn shoot P concentration at each stage
[c]AMF = Percentage of corn root length colonized by AMF at each stage
[d]n/s= Not significant

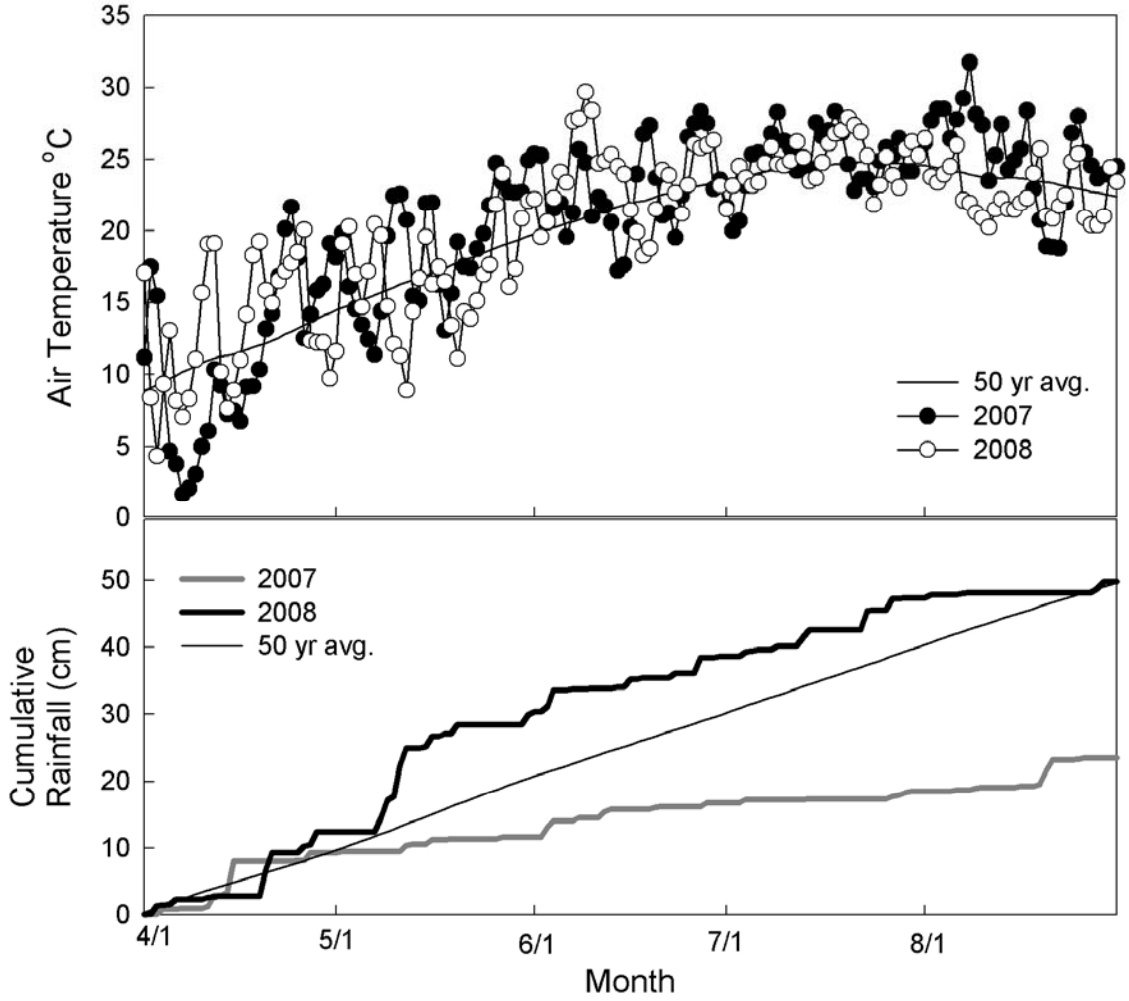

Figure 3.1. Air temperature and rainfall in Beltsville, MD from April through August during 2007 and 2008.

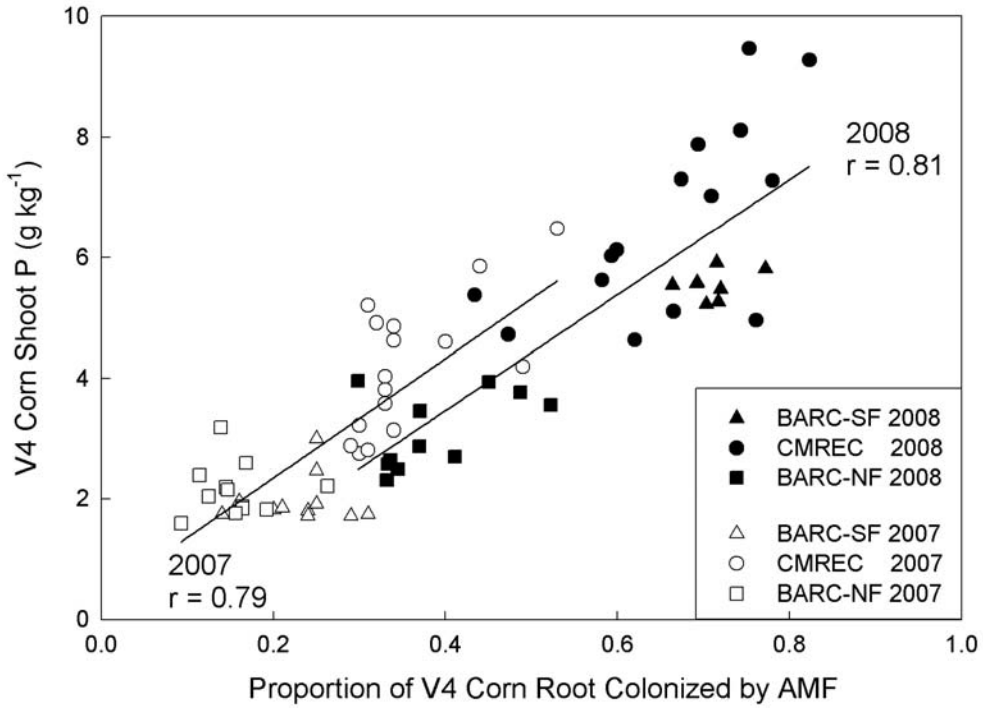

Figure 3.2. Significant correlation between AMF colonization of corn roots and corn shoot P concentration at the V4 stage in 2007 and 2008 (P<0.01).

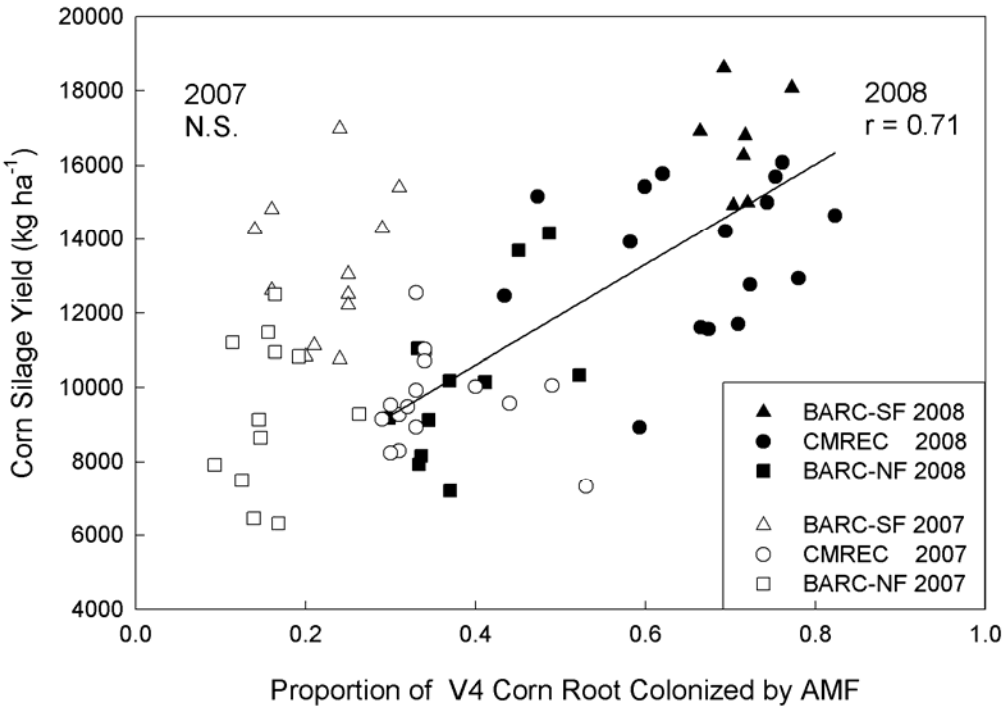

Figure 3.3. The correlation between AMF colonization of corn roots at the V4 stage and corn silage yield was significant in 2008 (P<0.01) but not in 2007.

Chapter 4. Forage Radish and Rye Cover Crop Effects on Phosphorus Cycling and Soil Test Phosphorus

Abstract

The growth and decomposition of cover crops influence nutrient cycling in the agroecosystem. Forage radish (*Raphanus sativus* L. var. *longipinnatus*) is a unique cover crop in terms of phosphorus (P) cycling because of its high tissue P concentration, rapid dry matter production in fall, and rapid decomposition in winter/spring. We compared P uptake by forage radish and cereal rye (*Secale cereale* L.) cover crops and measured soil test P stratification following cover crops in a 2 year study at each of 3 locations near Beltsville, Maryland. In each year the cover crops were planted in late August. Their P uptake ranged from 5.8 to 32 kg P ha^{-1} for forage radish shoots, from 3.5 to 7.0 kg P ha^{-1} for forage radish tap roots, and from 3.0 to 25 kg P ha^{-1} for rye shoots. Cover crops resulted in slight vertical stratification of soil test P at two sites. At one site, soil was sampled in 1 cm increments around radish root holes, revealing large increases in soil test P concentrations within 3 cm of forage radish root holes. At this site, the average Mehlich 3 soil test P in the 0 to 2.5 cm depth was 73 mg P kg^{-1} for the no cover crop treatment, 85 mg P kg^{-1} in the radish treatment > 5 cm from radish holes and 132 mg P kg^{-1} in the radish treatment < 3 cm from radish root holes. These findings have important implications for soil sampling and management practices aimed at measuring and managing P fertility and environmental risk of P pollution.

Keywords: forage radish, cereal rye, phosphorus, stratification, root hole

Introduction

Forage radish (*Raphanus sativus* L. var. *longipinnatus*) is being used in many parts of the world as a winter cover crop to alleviate soil compaction, reduce nitrate leaching, suppress weeds and control erosion (Weil and Kremen, 2007). In the Mid-Atlantic USA, forage radish is being used by an increasing number of dairy farmers as a cover crop between corn silage crops. Among the many unique characteristics of forage radish are its relatively high tissue phosphorus (P) concentration, rapid dry matter accumulation in the fall, and rapid residue decomposition in the spring. In addition, the forage radish produces a large fleshy taproot, typically 3 to 6 cm in diameter and 15 to 30 cm in length (Figure 4.1), that decays over winter to leave distinct holes in the surface soil in spring. These characteristics of forage radish cover crops present several potential opportunities and challenges for P management in the agroecosystem, including remediation of excessively high P soil, increased stratification of P at the soil surface, and improved fertility of low P soil.

Agricultural soil with excessively high P levels is common in the developed world and P transport from such soil to natural waters is one of the primary causes of eutrophication (Boesch et al., 2001; Sharpley et al., 2001). The concentration of P in soil can be reduced over time by eliminating the use of fertilizers containing P while continuing to remove P from the soil through harvested crops (Brown, 2006; Eghball et al., 2003). Cover crops may be harvested to feed to livestock directly as green chop or to make silage (Kratochvil et al., 2006). Harvesting a cover crop such as forage radish in addition to the main crops in a rotation could increase the amount of P removed from the soil each year, resulting in faster remediation of the soil to environmentally optimum P levels. The amount of manure or compost farmers can apply to fields is often severely

restricted under P-based nutrient management plans (Brown, 2006; Kratochvil et al., 2006). In some of these cases, the additional removal of P in harvested cover crops could increase the allowable manure application rates.

On the other hand, allowing cover crop residues to decompose at the soil surface, as is common practice in no-till agriculture, may lead to an accumulation of P in surface soil layers where it is susceptible to losses by runoff and erosion. Agricultural soil under no-till management commonly develops a high P concentration at the surface because crop residues and fertilizers are applied to the soil surface and P is relatively immobile in soil (Duiker and Beegle, 2006; Garcia et al., 2007; Sharpley, 2003; Weil et al., 1988). Cover crops that accumulate large quantities of P in their shoots may accentuate the stratification of soil P.

Increasing the plant-availability of soil P via biologically-based mechanisms has been studied as a means of improving soil fertility when soluble P fertilizers are not accessible to farmers. Members of the *Brassicaceae* plant family can solubilize recalcitrant forms of soil P by changing the rhizosphere pH (Grinsted et al., 1982; Hedley et al., 1982; Hinsinger and Gilkes, 1997; Marschner et al., 2007) and exuding organic acids (Hoffland et al., 1989; Shahbaz et al., 2006; Zhang et al., 1997). However, rotations or intercrops of Brassica species have shown little effect in improving availability of P for the companion or subsequent crop (Wang et al., 2007; Weil, 2000). When *Brassicas* are grown as a cash crop or as an intercrop, the soil P mobilized by the *Brassica* crop is removed at harvest or sequestered in plant tissue. A *Brassica* green manure or cover crop may be more effective in improving subsequent P availability because the P mobilized by the *Brassica* would be cycled back into the soil as the

residues decompose. Cavigelli and Thien (2003) found that P uptake of a sorghum (*Sorghum bicolor* L.) crop was positively correlated to the P uptake of a previous perennial forage green manure crop. In low P soil, *Brassica* species may prove advantageous as green manure crops due to their ability to accumulate greater levels of tissue P.

Other cover crops or green manures besides *Brassicas* may also increase P availability. Bah et al. (2006; 2003) found that green manures of two legume and one grass species increased P availability in an Ultisol and Reddy et al. (2005) found that incorporation of soybean (*Glycine max* L.) and wheat (*Triticum aestivum* L.) residues increased P availability in an Alfisol. In both cases, increases in P availability were attributed to a reduction in the soil's P sorption capacity because organic decomposition products from the green manures filled P sorption sites in the soil.

There have already been reports of increased soil test P following forage radish cover crops. Forage radish slightly increased soil test P compared to 3 other *Brassica* cover crops and a sorghum-sudan grass (*Sorghum bicolor* L. *X S. Sudanese* L.) cover crop at the 0 to 15cm depth range (Wang et al., 2008). In a study reported by Grove et al. (2007), soil test P increased in the 0 to 45cm depth range following three years of forage radish cover crops compared to rape (*Brassica napus* L.), cereal rye (*Secale cereale* L.), and no cover crop treatments.

This study has two objectives: 1) To quantify the amount of P accumulated in the tissue of forage radish and rye cover crops; and 2) To determine the effect of forage radish and rye cover crops on soil test P in bulk soil at different soil depths and in the soil immediately surrounding the holes created by forage radish tap roots.

Materials and Methods

Experimental Design

Experiments were conducted at three sites: the University of Maryland Central Maryland Research and Education Center (CMREC), the USDA Beltsville Agricultural Research Center North Farm (BARC-NF), and the USDA Beltsville Agricultural Research Center South Farm (BARC-SF). The experiments started in August 2006 and ended in August 2008. Location and soil properties of the individual sites are listed in Table 4.1.

At all sites a randomized complete block experimental design with four replicates was used. At all sites three cover crop treatments were included: forage radish, rye (*Secale cereale* L. cv. 'Wheeler'), and no cover crop. The no cover crop treatment was maintained weed free with herbicides. At BARC-SF plots were 3 m wide by 15 m long, at CMREC plots were 6 m wide by 12 m long, and at BARC-NF plots were 3 m wide by 9 m long.

Site-specific Management Operations

CMREC. Prior to the start of the experiment, a rotation of corn, followed by winter wheat (*Triticum aestivum* L.), and double crop soybeans was grown from 2005 to 2006. The soybean crop was mowed at a vegetative stage in early August 2006 and left to decompose as a source of nitrogen to promote cover crop growth on the sandy soil at this site. When the soybeans were mowed, the above ground dry matter contained 56 kg N ha^{-1} with a C/N ratio of 13. The field had been managed using no-till practices since the fall of 2003 when it was last chisel plowed. Cover crops were planted on 12 September 2006 using a no-till drill with 16 cm row spacing. Rye was seeded at a rate of 135 kg ha^{-1} and forage radish was planted at a rate of 14 kg ha^{-1}. The forage radish cover

crop was killed naturally when temperatures dropped below -4 ^{o}C in January and the rye cover crop was killed when all plots in the experiment were sprayed with paraquat dichloride (0.68 L ha^{-1} a.i.) and 2,4-D (2,4-dichlorophenoxyacetic acid) (1.05 L ha^{-1} a.i.) on 10 April 2007.

Corn (Pioneer 38B84, glyphosate tolerant) was planted at a rate of 74,000 seeds ha^{-1} with 75 cm row spacing on 23 April 2007. At planting, a 30% UAN solution was applied in a band 5 cm to the side of the seed furrow at a rate of 22 kg N ha^{-1}. Glyphosate (1.85 L ha^{-1} a.i.) was sprayed on 7 May 2007 to control weeds. The corn was side dressed with 112 kg N ha^{-1} as 30% UAN solution knifed in to a depth of 10 cm between every second corn row on 6 June 2007. The corn was harvested as silage on 16 August 2007.

To prepare the field for the second year of cover crops, weeds were killed with glyphosate (1.85 L ha^{-1} a.i.) and 22 kg N ha^{-1} as 30% UAN solution was applied as a starter fertilizer for the cover crops to ensure adequate growth. Cover crops were planted on 28 August 2007 in the same manner as the year before. Weeds in the no cover crop plots were controlled with glyphosate (1.85 L ha^{-1} a.i.) on 18 September 2007. The forage radish cover crop was killed naturally when temperatures dropped below -4 ^{o}C in January and the rye cover crop was killed when all plots in the experiment were sprayed with paraquat dichloride (0.68 L ha^{-1} a.i.) and 2,4-D (1.05 L ha^{-1} a.i.) on 12 April 2008.

On 16 April 2008, corn was planted using the same practices as in 2007. At planting, a 30% UAN solution was applied in a band 5 cm to the side of the seed furrow at a rate of 22 kg N ha^{-1}. Glyphosate (1.85 L ha^{-1} a.i.), atrazine (0.42 L ha^{-1} a.i.), s-metolachlor (0.43 L ha^{-1} a.i.), and mesotrione (2-[4-(methylsulfonyl)-2-nitrobenzoyl]$^{-1}$,3-

cyclohexanedione) (0.05 L ha^{-1} a.i.) were applied on 16 May 2008 to control weeds. The corn was side dressed with 112 kg N ha^{-1} as 30% UAN solution knifed in to a depth of 10 cm between every second row on 6 June 2007. The corn was harvested as silage on 12 August 2008.

BARC-NF. Prior to the start of the experiment, potatoes (*Solanum tuberosum* L.) and green beans (*Phaseolus vulgaris* L.) were grown in 2005 and 2006 respectively. A cover crop of rye was established in the fall of 2005. The field had a long history of conventional tillage practices. Prior to the start of the experiment the field was chisel plowed in October 2005 and moldboard plowed in June 2006 and again in August 2006. The field was disked following each of these plowings. Once the experiment started, however, the field was managed using no-till practices to minimize soil disturbance.

Cover crops were planted on 31 August 2006 using a no-till drill with 16 cm row spacing. Rye was seeded at a rate of 135 kg ha^{-1} and forage radish was planted at a rate of 14 kg ha^{-1}. The forage radish cover crop was killed naturally when temperatures dropped below -4 ^{o}C in January and the rye cover crop was killed when all plots in the experiment were sprayed with paraquat dichloride (0.68 L ha^{-1} a.i.) on 11 April 2007.

Corn (Pioneer 38B84, glyphosate tolerant) was planted at a rate of 74,000 seeds ha^{-1} with 75 cm row spacing on 24 April 2007. Granular ammonium nitrate was applied in a band 5 cm to the side of the seed furrow and 5 cm deep at a rate of 22 kg N ha^{-1}. Glyphosate (1.85 L ha^{-1} a.i.) was sprayed on 9 May 2007 to control weeds. The corn was side dressed with 112 kg N ha^{-1} as 30% UAN solution dribbled on the soil surface between rows on 20 June 2007. The corn was harvested as silage on 16 August 2007.

To prepare the field for the second year of cover crops, weeds were killed with glyphosate (1.85 L ha^{-1} a.i.) and 22 kg N ha^{-1} as 30% UAN solution was applied as a starter fertilizer for the cover crops to ensure adequate growth. Cover crops were planted on 27 August 2007 in the same manner as the year before. Weeds in the no cover crop plots were controlled with glyphosate (1.85 L ha^{-1} a.i.) on 3 October 2007. The forage radish cover crop was killed naturally when temperatures dropped below -4 ^{o}C in January and the rye cover crop was killed when all plots in the experiment were sprayed with glyphosate (1.85 L ha^{-1} a.i.) and 2,4-D (1.05 L ha^{-1} a.i.) on 16 April 2008.

On 7 May 2008 corn was planted using the same practices as in 2007. Granular ammonium nitrate was applied in a band 5 cm to the side of the seed furrow and 5 cm deep at a rate of 22 kg N ha^{-1}. Glyphosate (1.85 L ha^{-1} a.i.) was sprayed on 18 June 2008 to control weeds. The corn was side dressed with 112 kg N ha^{-1} as 30% UAN solution dribbled on the soil surface between rows on 17 June 2008. The corn was harvested as silage on 12 August 2008.

BARC-SF. Prior to the start of the experiment, sweet corn and soybeans were grown in 2004 and 2005, respectively. Following the harvest of soybeans in the fall of 2005, the field remained fallow until the cover crop experiment was planted in August 2006. The field had a long history of conventional tillage practices. In 2006, the field was moldboard plowed in May and disked in June and July prior to the start of the cover crop experiment in August. Once the experiment started, however, the field was managed using no-till practices. On 30 August 2006 fertilizers were broadcast applied at rates of 84 kg ha^{-1} of K as potassium chloride, 17 kg ha^{-1} of P as triple super phosphate, and 62 kg ha^{-1} of N as urea.

Cover crops were planted on 31 August 2006 using a no-till drill with 16 cm row spacing. Rye was seeded at a rate of 135 kg ha^{-1} and forage radish was planted at a rate of 14 kg ha^{-1}. The forage radish cover crop was killed naturally when temperatures dropped below -4 ^{o}C in January and the rye cover crop was killed when all plots in the experiment were sprayed with glyphosate (N-(phosphonomethyl)glycine) applied at a rate of 1.85 L ha^{-1} active ingredient (a.i.) on 10 April 2007.

Corn (Pioneer 38B84, glyphosate tolerant) was planted at a rate of 74,000 seeds ha^{-1} with 75 cm row spacing on 24 April 2007 using a no-till planter. Granular ammonium nitrate was applied in a band 5 cm to the side of the seed furrow and 5 cm deep at a rate of 22 kg N ha^{-1}. The corn was side dressed with 112 kg N ha^{-1} as 30% urea ammonium nitrate (UAN) solution dribbled on the soil surface between rows on 7 June 2007. Glyphosate (1.85 L ha^{-1} a.i.) was sprayed on 7 June 2007 to control weeds. The corn was harvested as silage on 16 August 2007.

To prepare the field for the second year of cover crops, weeds were killed with glyphosate (1.85 L ha^{-1} a.i.) and 22 kg N ha^{-1} as 30% UAN solution was applied as a starter fertilizer for the cover crops to ensure adequate growth. Cover crops were planted on 27 August 2007 in the same manner as the year before. Weeds in the no cover crop plots were controlled with glyphosate (1.85 L ha^{-1} a.i.) on 4 October 2007 and paraquat dichloride (1,1'-dimethyl-4,4'-bipyridinium dichloride) (0.68 L ha^{-1} a.i.) on 5 November 2007. The forage radish cover crop was killed naturally when temperatures dropped below -4 ^{o}C in January and the rye cover crop was killed when all plots in the experiment were sprayed with glyphosate (1.85 L ha^{-1} a.i.) on 16 April 2008.

On 7 May 2008 corn was planted using the same practices as in 2007. Granular ammonium nitrate was applied in a band 5 cm to the side of the seed furrow and 5 cm deep at a rate of 22 kg N ha^{-1}. The corn was side dressed with 112 kg N ha^{-1} as 30% UAN solution dribbled on the soil surface between rows on 10 June 2008. Glyphosate (2.77 L ha^{-1} a.i.), atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) (1.49 L ha^{-1} a.i.), and s-metolachlor (acetamide, 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy^{-1}-methylethyl]-,(S)) (1.61 L ha^{-1} a.i.) were sprayed on 10 June 2008 to control weeds. The corn was harvested as silage on 12 August 2008.

Cover Crop Dry Matter Sampling

The shoots and fleshy tap roots of the forage radish cover crop were sampled near the time of maximum dry matter accumulation in the late fall. The shoots of the rye cover crop were sampled in the spring, prior to being killed by herbicides. Exact dates of cover crop sampling are listed in Table 4.3. Cover crop samples were obtained by removing plant parts from two 0.25 m^2 quadrats in each plot. At CMREC and BARC-SF, both the shoots and the fleshy tap roots of forage radish were sampled. Forage radish plants rooted within the quadrat were pulled from the soil by hand to collect the tap roots. Roots and shoots were separated in the field. At BARC-NF forage radish roots were not sampled. For rye, only shoots were sampled at all sites. All plant parts were washed to remove attached soil prior to drying in a forced draft oven at 60 ºC for a minimum of 7 days. Samples were weighed then ground and sieved to <2 mm particle size and stored in sealed polyethylene vials.

Plant Tissue Phosphorus Analysis

Plant tissue phosphorus concentration was determined by ashing 0.4 g of each tissue sample in a muffle furnace at 550 ºC for five hours. The ash was dissolved in 40 mL of 0.3 M HCl and filtered through Whatman No. 42 filter paper (Whatman International, Maidstone, UK). The phosphorus concentration of the filtrate was measured colorimetrically using the vanadomolybdophosphoric acid method (Kuo, 1996) on a spectrophotometer set to 420 nm (DU720, Beckman-Coulter, Inc., Fullerton, CA).

Soil Sampling

Bulk soil samples were collected from each plot in the spring of 2007 and 2008. A 1.85 cm diameter soil probe was used to collect 6 cores from random locations within each plot. In the radish plots, locations within 5 cm of a radish hole were excluded from sampling. In 2007, samples were collected on 24 April, 10 April, and 5 April at CMREC, BARC-NF, and BARC-SF, respectively. In 2008, samples were collected on 15 May, 25 April, and 15 May at CMREC, BARC-NF, and BARC-SF respectively. Each core was divided into depth segments of 0 to 2.5 cm, 2.5 to 10 cm, and 10 to 20 cm. The 6 cores were pooled to create a composite sample of each depth range. Soil samples were stored under refrigeration in sealed bags for <24 hours before being air-dried. After drying, soil samples were crushed and sieved through a 2 mm screen and stored in sealed polyethylene vials.

Small-scale soil sampling was conducted around the perimeter of an individual forage radish tap root hole in three replications of the experiment on 8 May 2008 at the BARC-SF site. Soil samples were collected by carefully removing soil from around the circumference of the root holes in 1 cm wide by 1 cm deep sections using a stainless steel spatula (Figure 4.2). The 1 cm wide by 1 cm deep sections were obtained to a depth of 5

cm from the surface and to a width of 3 cm from the outer edge of the root hole. These soil samples were air-dried immediately, then crushed and sieved through a 2 mm screen and stored in sealed polyethylene vials.

Soil Phosphorus Analysis

Mehlich 3 soil test P concentration was selected as the method to measure soil P because it is widely used in soil fertility studies as a predictor of plant available P and in environmental studies as a predictor of water soluble P, desorbable P (Fe oxide strip P), and total sorbed P (oxalate P) (Sims et al., 2002). Soil samples were analyzed for Mehlich 3 extractable phosphorus (Mehlich, 1984; Sims, 2000) using 2 g of soil and 20 mL of extracting solution. The extracting solution was filtered through Whatman No. 42 filter paper (Whatman International, Maidstone, UK). The phosphorus concentration in the extracting solution was measured colorimetrically using the ascorbic acid method (Kuo, 1996) on a spectrophotometer set to 880 nm (DU720, Beckman-Coulter, Inc., Fullerton, CA).

Statistical Analysis

Cover crop dry matter, tissue P concentration, and P uptake data were analyzed by ANOVA in the Mixed procedure of SAS (SAS Institute, Cary, NC). To maximize the power of the analysis, data from all sites and years were pooled in the ANOVA. Sites and blocks were considered random factors and cover crop and year were considered fixed factors. Year was treated in the experimental design as a split-plot factor within cover crop main plots. When the ANOVA indicated a statistically significant treatment effect ($P<0.05$), mean comparisons were made using Fisher's Least Significant Difference test.

Mehlich 3 soil test P data from bulk soil cores were also analyzed by ANOVA in the Mixed procedure of SAS, but sites were analyzed separately to reduce the number of interactions in the model. Year was treated in the experimental design as a split-plot factor within cover crop main plots and soil depth was treated as a repeated measure within year split-plots. The appropriate covariance structure for the repeated measurement was determined based on the covariance structure which resulted in the lowest AIC fit statistic in SAS. When the ANOVA indicated a statistically significant treatment effect ($P<0.05$), mean comparisons were made using Fisher's Least Significant Difference test.

Mehlich 3 soil test P data from the radish root hole sampling were analyzed by ANOVA in the Mixed procedure of SAS. Each of the three radish root holes was treated as a replicate in the experimental design. The position of each sample point in relation to the soil surface and the wall of the root hole was treated as a repeated measurement within a radish hole replicate. Spatial autocorrelation between sampling positions around the radish hole was addressed by using an anisotropic exponential spatial covariance structure for the repeated measurement in the ANOVA. Mean values from each sampling point around the radish hole were compared to two control values using Dunnett's test. One control was the soil test P value of the no cover crop plots and the other control was the soil test P value of the bulk soil in radish plots between rows.

Results and Discussion

Cover crop dry matter and P content

Dry matter production, tissue P concentration, and shoot P uptake of cover crops varied among sites and between years (Table 4.2). At CMREC in both years, cover crops

produced less dry matter than at the other sites (Table 4.3). For forage radish, this was due to a late planting date in 2006 and because of nitrogen deficiencies on this loamy sand soil in both years, which were identified based on visual observations of chlorosis of the oldest leaves. The low dry matter production of rye at CMREC may have also been related to nitrogen deficiencies, although chlorotic leaves were not observed. The soil at CMREC is a coarser texture than at the other sites which may have caused residual nitrogen to leach beyond the rooting zone of the cover crops more readily than at the other sites. Also, the soil at CMREC has less organic matter than the other sites, so is likely to supply less mineralized N during the growth of cover crops in the fall and spring. Finally, the mowed soybeans in 2006 may have provided insufficient nitrogen for cover crop growth. At BARC-NF and BARC-SF, rye and forage radish growth was normal and achieved shoot dry matter production over 4,000 kg ha^{-1} in all years and over 7,000 kg ha^{-1} of rye at BARC-NF in 2008 and BARC-SF in 2007 (Table 4.3).

Tissue P concentrations also varied among sites and between years (Table 4.2). Radish shoot P concentration was greater than rye shoot P concentration at all sites and years (Table 4.3). The P concentration of the radish roots was sometimes greater than, sometimes less than, and sometimes the same as the P concentration of radish shoots. Across all site years, the mean tissue P concentrations were 4.5 g P kg^{-1} for radish roots, 5.3 g P kg^{-1} for radish shoots, and 2.7 g P kg^{-1} for rye shoots.

Total P uptake by cover crops is influenced by both the tissue P concentration and by total dry matter accumulation. However, in this study, total P uptake was more closely associated with dry matter production of the cover crops (r=0.84) than with tissue P concentrations (r=0.49). Despite the lower shoot P concentration of rye compared to

radish, in cases where rye accumulated over 7,000 kg ha^{-1}, such as at BARC-NF in 2008 and at BARC-SF in 2007, total P uptake by rye shoots was equivalent to that of radish shoots (Table 4.3). Across all site years, the mean P uptake by cover crops was 20 kg P ha^{-1} for radish shoots, 5.0 kg P ha^{-1} for radish roots, and 12.7 kg P ha^{-1} for rye shoots. At BARC-SF, where conditions were optimal for radish growth, radish roots took up only 20% as much P as the shoots did. At CMREC, where radish growth was limited by planting date and nitrogen, radish roots took up similar amounts of P as radish shoots in 2007, and 30% as much P as radish shoots in 2008.

Although we did not find any other studies reporting P uptake of forage radish cover crops in the literature, there are several reports of the P uptake of oilseed radish (*Raphanus sativus* L. var. *oleiferus*), a close relative of forage radish with similar characteristics. Wang et al. (2008) reported combined root and shoot dry matter production, tissue P concentration and total P uptake for oilseed radish of 6,262 kg ha^{-1}, 6.8 g P kg^{-1}, and 42.7 kg P ha^{-1}, respectively, in a field with an optimum level of soil test P. In a field with low soil test P levels, Brown et al. (2008) found shoot dry matter production, tissue P concentration and total P uptake for oilseed radish to be 5,681 kg ha^{-1}, 2.2 g P kg^{-1}, and 12.5 kg P ha^{-1}, respectively, when no P fertilizer was applied to the cover crop. However, when 168 kg P ha^{-1} of P fertilizer was added, shoot dry matter production, tissue P concentration and total P uptake increased to 9,090 kg ha^{-1}, 5.5 g P kg^{-1}, and 50 kg P ha^{-1}, respectively.

Brown (2006) reported that after three years of double cropping a winter cereal forage with corn silage in Idaho, soil test P levels declined 50% more compared to only growing and harvesting corn silage. In that study, winter cereal forage crops removed

between 10 and 30 kg P ha^{-1} annually, similar to the quantity of P we found forage radish and rye cover crops to accumulate. The average cumulative P harvest removal over the 3 year study for the double cropped system was 175 kg P ha^{-1}. A study in Maryland by Kratochvil et al. (2006), however, found that after three years of a cereal rye silage and corn silage forage system, soil test P levels did not decline despite cumulative P harvest removals between 95 and 135 kg P ha^{-1}. Aside from the greater P removal in the Idaho study, a major difference between these studies is that the Idaho study used mineral fertilizer to establish the initial soil test P level while the Maryland study used manure. In the Maryland study, mineralization of organic P in manure may have replaced mineral P removed by harvest, resulting in no decline in soil test P. Predicting how P harvest removals will influence changes in soil test P should also take into account "hidden" additions of mineral P such as occur from mineralization of organic matter.

Our findings suggest that for the purpose of P removal, cover crops should be managed to achieve maximum dry matter production. This is similar to the findings of Eghball et al. (2003), who tested a number of corn hybrids and soybean cultivars for P removal potential, and found that P removal in grain was linearly correlated with grain yield. In Maryland's climate, maximizing dry matter production for forage radish requires a planting date before September 1 and an adequate supply of available nitrogen. For rye, allowing its growth to continue as late into spring as possible will maximize dry matter production and can result in P removal rates as great or greater than forage radish. Forage radish, which reaches its full growth potential by November, offers the advantage of significant P removal in a shorter time frame, but has the disadvantage of requiring an early planting date. Although the fleshy tap roots of forage radish can easily be removed

from the soil by hand, the additional P contained in the tap roots is small compared to that contained in shoots and the removal of the roots would result in only minimal P removal.

Bulk Soil P

At CMREC, the cover crop X depth X year interaction was significant (Table 4.2). In 2007, cover crops had no effect on soil test P levels at any of the depth ranges (Table 4.4). Soil test P at the 0 to 2.5 cm and 2.5 to 10 cm depths was less than the soil below it for all cover crop treatments. This stratification of soil test P may be because during the period of time that the field had been in no-till management prior to this experiment, no P fertilizer had been applied and crops extracted greater quantities of P at the 0 to 10 cm depth as compared to the 10 to 20 cm depth. In 2008, soil test P levels were still markedly stratified following rye and no cover crop. However, following radish, soil test P at the 0 to 2.5 cm depth had significantly increased from the prior year.

At BARC-NF, the cover crop X year and depth X year interactions were significant (Table 4.2). There was no difference in soil test P between cover crops in either 2007 or 2008 (Table 4.4). However, soil test P declined from 2007 to 2008 in the rye cover crop treatment but not in the other treatments. This decline may be a result of P immobilization, which can be caused by plant residues with a P concentration of less than 2 g P kg^{-1} (Cavigelli and Thien, 2003). There was also a change in the distribution of soil test P with depth between years. From 2007 to 2008, soil test P declined in the 10 to 20 cm depth while it remained constant at the depths above 10 cm. This may have been because 2007 was a dry summer and most of the P taken up by corn roots was from deeper soil layers where there was more moisture.

At BARC-SF, cover crop X depth and depth X year interactions were significant (Table 4.2). Averaged across years, a forage radish cover crop resulted in greater soil P at the 0 to 2.5 cm depth range compared to no cover crop while a rye cover crop resulted in an intermediate soil P level that was not statistically different from either radish or no cover crop (Table 4.4). Soil test P also changed with depth following forage radish and rye cover crops, with greater levels of P at the 0 to 2.5 cm and 2.5 to 10 cm depths than at the 10 to 20 cm depth. In 2008, soil test P levels were less than in 2007 for all depth ranges.

Radish Root Hole Soil P

Mehlich 3 soil test P levels proximal to radish holes (within 3 cm of the soil surface and within 1 cm of the root channel) were always significantly greater than the soil test P level in the no cover crop treatment (Table 4.5). Compared to the bulk soil collected from 0 to 2.5 cm depth in radish plots, soil test P levels around the radish hole always were greater within 1 cm of the of the root hole and within 2 cm of the surface. In general, soil test P levels were greatest at the surface of the soil and closest to the wall of the root channel and declined with depth from the surface and with distance from the root hole.

The results of the intensive radish root hole sampling indicate that areas around radish root holes are enriched in phosphorus compared to both soil that hasn't been cover cropped and the bulk soil between radish rows. This finding has several implications for P fertility, P pollution, and soil sampling methodology.

Bulk soil samples collected from forage radish plots but away from radish holes resulted in an average soil test P of 85 mg P kg^{-1} at the 0 to 2.5 cm depth. This value is

considered in the optimum range for crop productivity, but is well below the 150 mg P kg^{-1} threshold where soils are considered to present a risk of environmental P pollution (Sims et al., 2002). Sampling within 3 cm of radish holes, however, resulted in soil test P levels in the surface 1 cm ranging from 137 to 142 mg P kg^{-1}, with some individual samples as high as 177 mg P kg^{-1}. A forage radish cover crop increased the P concentration of soil that was proximal to root holes to levels that were close to or above the environmental risk threshold, but this was not detected by sampling soil between radish rows to a depth of 0 to 2.5 cm. Soil sampling protocols often specify avoiding areas of soil that are known to be abnormal, as some might consider a radish root hole to be. The area within 3 cm of a radish hole can account for a large portion of a field, however (Figure 4.3). Recommended seeding rates of forage radish often result in approximately 80 radish root holes per square meter. Assuming the radius of a radish hole to be 2 cm, the area within 3 cm of the wall of a radish hole will account for about 60% of the field ((5 cm * 5 cm * 3.14 * 80) / (100 cm* 100 cm) = 0.628).

Similar lateral heterogeneity in soil test P has been found by others. Duiker and Beegle (2006) found a heterogenous lateral distribution of soil test P when fertilizer applications were injected or dribbled in bands in the same location between crop rows over the course of 25 years. Similar results were also found by Mallarino and Borges (2006) after 4 years of injecting fertilizer bands. In both studies, soil test P was approximately 40% greater in banded areas at 0-5 cm depth in a no-till system. This difference between low and high P soil zones across the row was of similar magnitude to the difference we found between radish holes and bulk soil.

For the purposes of soil fertility sampling, Duiker and Beegle recommended that to avoid underestimating fertilizer requirements, soil samples should be taken only from the low P soil zones between the bands. For the purposes of monitoring the potential for environmental losses of P, however, they recommended sampling from known zones of high P soil. Mallarino and Borges, on the other hand, recommended that soil fertility samples should be taken from the fertilizer bands when their location is known. When the location of bands is unknown, they recommended sampling from random locations and increasing the number of cores collected in the composite sample. Further research is necessary to determine how heterogeneity of soil test P levels affects crop response and what the best soil sampling procedure is. Until then, when taking soil fertility samples from a field with a recent history of forage radish cover cropping, we recommend that cores be taken from random locations within a field and that a larger number of cores should be collected in the composite sample.

The finding that increased soil test P levels occur in proximity to the radish root hole and decline with distance may also explain why at CMREC and BARC-NF, bulk soil sampled between radish rows did not have greater soil test P levels compared to the no cover crop treatment. We likely missed the effect by sampling in the wrong location.

Bulk soil samples did, however, show that slight but statistically significant stratification of soil test P occurred after rye and forage radish cover crops at BARC-SF. At CMREC, forage radish redistributed soil test P which was originally stratified with greater concentrations as depth increased. These small differences between depths could be declared statistically significant while larger differences between cover crop treatments could not because comparisons within a repeated measure generally are more

powerful than comparisons between main plots. The magnitude of the stratification in bulk soil that we found following two years of cover crops, however, is quite small compared to the stratification effect that occurs in no-till fields when P fertilizer is added to the soil surface (Garcia et al., 2007; Mallarino and Borges, 2006; Sharpley, 2003).

The effect forage radish cover crops have on the spatial distribution of soil P may also have implications for P availability to crops. Forage radish increases soil test P levels in the vicinity of its root hole, possibly through deposition of high P residue, by mobilizing P with root exudates, or because organic matter reduces the P sorption capacity of the soil. Following a forage radish cover crop, the roots of main crops have been shown to preferentially grow in previously formed forage radish root channels (Williams and Weil, 2004), so the P availability along these channels may be more important for crop nutrition than the P availability in bulk soil. However, the soils examined in this study already had optimum soil test P levels and no correlations were found between soil test P and crop growth or yield (data not shown). Despite this, the finding of increased soil test P in the vicinity of radish root holes does justify further research in low P soils.

Conclusions

We found that shoots of both forage radish and rye cover crops have the potential to take up significant quantities of P when they are managed for maximum dry matter production. Based on these findings, harvesting cover crops in addition to main crops would increase P removal from the soil, leading to faster declines in soil test P. Increasing annual crop removal of P would also allow farmers to increase the rate of manure additions in P-based nutrient management plans.

Allowing forage radish residues to decompose on the soil surface can result in large increases in soil test P in the vicinity of the radish root holes. More importantly, these large increases were not detected when soil cores were collected from at least 5 cm away from root holes. Soil sampling techniques that systematically avoid radish root holes may underestimate soil test P levels. Future research should investigate the implications of these findings for environmental P management in high P soils and for P fertility management in low P soils.

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Table 4.1. Selected properties of experimental sites and soils.

	Site		
	CMREC	BARC-NF	BARC-SF
Location	Greenbelt, MD	Beltsville, MD	Beltsville, MD
Latitude	39° 00' 42" N	39° 00' 51" N	39° 02' 01" N
Longitude	76° 49' 54" W	76° 56' 31" W	76° 55' 53" W
Hectares	0.57	0.40	0.93
Slope (%)	4	4	0.5
Soil Series	Downer	Hammonton	Codorus
Soil Taxonomy	Coarse-loamy, siliceous, semiactive, mesic Typic Hapludult	Coarse-loamy, siliceous, semiactive, mesic Aquic Hapludult	Fine-loamy, mixed, active, mesic Fluvaquentic Dystrudept
Surface (0-10 cm) soil properties			
pH_w	5.9 (0.30)[a]	5.6 (0.08)	6.9 (0.13)
Organic Matter[b] (g $100g^{-1}$)	1.1 (0.14)	1.3 (0.18)	1.6 (0.05)
Mehlich 3 P (mg kg^{-1})	88 (5.31)	102 (5.98)	98 (1.72)
Sand[c] (g $100g^{-1}$)	78	54	50
Silt (g $100g^{-1}$)	17	42	38
Clay (g $100g^{-1}$)	5	4	12

[a]Where multiple samples were measured, standard error is listed in parentheses; (*n*=4)
[b]Soil Organic matter by loss on ignition
[c]Particle size analysis by the hydrometer method

Table 4.2. Significance of treatment effects on cover crop dry matter, cover crop tissue P concentration, cover crop P uptake, and bulk soil Mehlich 3 soil test P. To maximize power in the analysis of cover crops, all site years were pooled into a single ANOVA. In the analysis of soil test P, sites were analyzed separately. Values indicate the probability of a greater F-value (α=0.05).

		Cover Crops		
Source of variation	df	Dry Matter	Tissue P conc.	P uptake
Cover	15	<0.0001	<0.0001	<0.0001
Site	0	.	.	.
Site X Cover	15	<0.0001	<0.0001	<0.0001
Year	24	0.6200	0.0002	0.0300
Year X Cover	24	0.0632	<0.0001	0.1665
Year X Site	24	<0.0001	<0.0001	0.2681
Year X Site X Cover	24	<0.0001	<0.0001	<0.0001
		Soil Test P		
		Site		
Source of variation	df	CMREC	BARC-NF	BARC-SF
Cover	6	0.3564	0.5121	0.1785
Depth	44	<0.0001	0.7024	<.0001
Cover X Depth	44	0.0541	0.0625	0.0272
Year	44	0.0561	0.0131	<.0001
Cover X Year	44	0.2144	0.0134	0.2822
Depth X Year	44	0.1523	0.0357	0.0170
Cover X Depth X Year	44	0.0164	0.7651	0.5700

Table 4.3. Cover crop dry matter production, tissue P concentration, and P uptake measured for forage radish in fall and for rye in spring. Within site and year, means followed by different letters are significantly different (F-protected LSD, P<0.05).

Cover Crop Tissue	DM (kg ha^{-1})	P conc. (g P kg^{-1})	P uptake (kg P ha^{-1})	Planting Date	Sampling Date
CMREC 2007					
Radish Root	1157a	6.1c	7.0b	12 Sept. '06	15 Nov. '06
Radish Shoot	1306ab	4.5b	5.8b	12 Sept. '06	15 Nov. '06
Rye Shoot	1711b	1.8a	3.0a	12 Sept. '06	01 April '07
BARC-NF 2007					
Radish Shoot	5583a	5.7b	32b	31 Aug. '06	29 Nov. '06
Rye Shoot	4177a	1.7a	6.9a	31 Aug. '06	07 April '07
BARC-SF 2007					
Radish Root	936a	3.8ab	3.5a	31 Aug. '06	15 Nov. '06
Radish Shoot	4282b	4.1b	18b	31 Aug. '06	15 Nov. '06
Rye Shoot	7345c	3.4a	25c	31 Aug. '06	01 April '07
CMREC 2008					
Radish Root	1084a	4.1b	4.5a	28 Aug. '07	09 Nov. '07
Radish Shoot	2629c	5.4c	14b	28 Aug. '07	09 Nov. '07
Rye Shoot	1546b	2.1a	3.3a	28 Aug. '07	07 Mar. '08
BARC-NF 2008					
Radish Shoot	4642a	5.7b	26a	27 Aug. '07	20 Nov. '07
Rye Shoot	7062b	3.2a	22a	27 Aug. '07	11 April '08
BARC-SF 2008					
Radish Root	1235a	4.1a	5.1a	27 Aug. '07	09 Nov. '07
Radish Shoot	4026b	6.3b	25c	27 Aug. '07	09 Nov. '07
Rye Shoot	4117b	3.9a	16b	27 Aug. '07	07 Mar. '08

Table 4.4. Mehlich 3 soil test P (mg P kg^{-1}) of bulk soil samples collected in the spring following rye cover crop termination. In the case of radish plots, subsamples were collected no closer than 5 cm from a root hole. Sites were analyzed separately. Within each indicated interaction, means followed by different letters are significantly different according to: a, b, c – means compared within the column; g, h – means compared within the row; x, y – means compared between years (P<0.05, F-protected LSD).

		Cover X Depth			Year X Depth	
BARC-SF	Depth	Radish	Rye	No Cover	2007	2008
	0 – 2.5 cm	95 c h	89 b gh	78 a g	93 c h	81 b g
	2.5 – 10 cm	89 b g	88 b g	76 a g	88 b h	81 b g
	10 – 20 cm	84 a g	83 a g	75 a g	84 a h	77 a g

		Cover X Depth X Year					
		2007			2008		
CMREC	Depth	Radish	Rye	No Cover	Radish	Rye	No Cover
	0 – 2.5 cm	62 a g x	61 a g x	70 a g x	74 ab h y	61 a g x	70 a gh x
	2.5 – 10 cm	65 a g x	66 a g x	66 a g x	69 a g x	68 a g x	74 a g y
	10 – 20 cm	85 b g x	85 b g x	87 b g x	79 b g x	83 b gh x	94 b h x

		Cover X Year			Depth X Year		
BARC-NF	Year	Radish	Rye	No Cover	Depth	2007	2008
	2007	95 a g	92 b g	93 a g	0 – 2.5 cm	92 a g	93 b g
	2008	95 a g	84 a g	92 a g	2.5 – 10 cm	94 a g	91 ab g
					10 – 20 cm	95 a g	87 a h

Table 4.5. Mehlich 3 P extracted from soil sampled in 1 cm increments around radish root holes at BARC-SF in May 2008. The sampling extended 5 cm in depth from the surface and 3 cm horizontally from the wall of the root hole. Asterisks represent a significant difference from a no cover crop control soil, collected from the 0-2.5 cm depth, which had a mean value of 73 mg P kg^{-1}. Letters represent a significant difference from a radish control soil, collected from 0-2.5 cm depth and not within 5 cm of a radish hole, which had a mean value of 85 mg P kg^{-1}. (* or b: P<0.05, ** or a: P<0.01, Dunnett's Test)

	Distance from Root Hole		
	0-1cm	1-2cm	2-3cm
Depth	———	mg P kg^{-1}	———
0-1 cm	142**a	142**a	137**a
1-2 cm	140**a	125**a	123**a
2-3 cm	133**a	114**b	108**
3-4 cm	121**a	104*	98
4-5 cm	115**b	98	95

Figure 4.1. Forage radish taproots typically measure 3 to 6 cm in diameter and 15 to 30 cm in length.

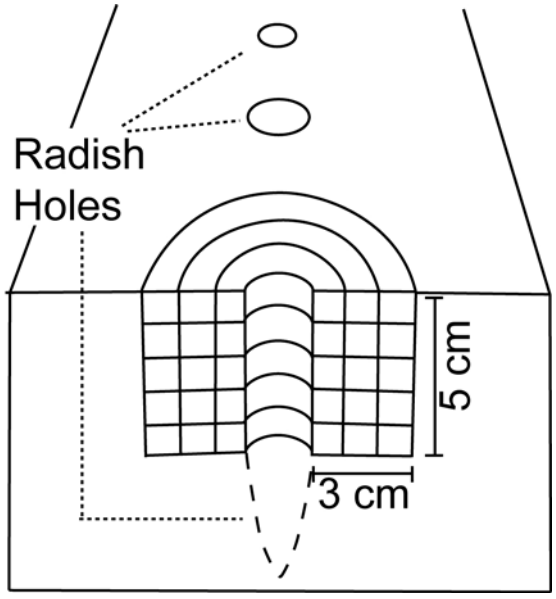

Figure 4.2. A cross section of the small-scale soil sampling conducted around the perimeter of individual radish holes. Soil was sampled in 1 cm wide by 1 cm deep increments around the perimeter of radish roots holes, extending 3 cm from the edge of the root hole and 5 cm from the surface of the soil.

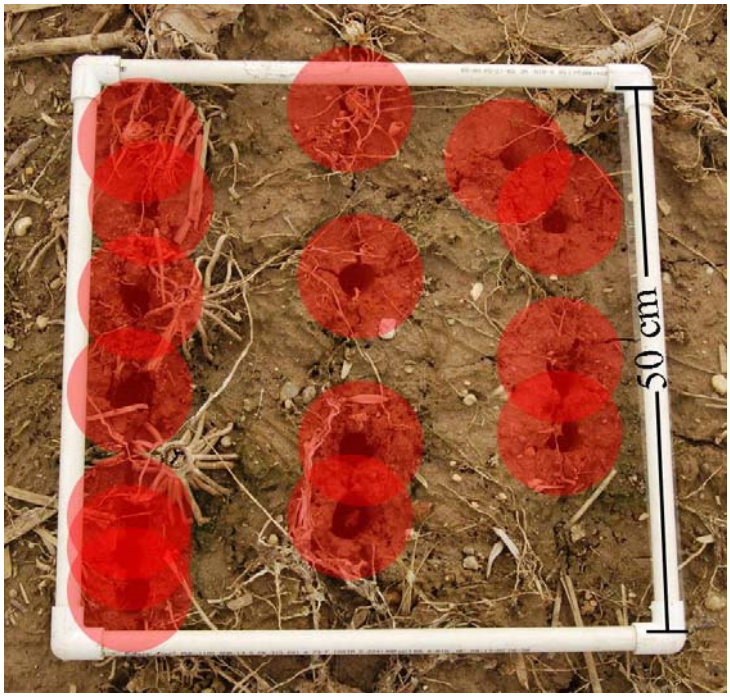

Figure 4.3. Approximately 50% of the area in this 0.25 m^2 quadrat is within 3 cm of the edge of a radish hole.

Chapter 5. Overall Conclusions

Several important findings have resulted from this study. First, we found that forage radish cover crops caused no impediment to subsequent colonization of corn roots by arbuscular mycorrhizal fungi (AMF). In all cases, AMF colonization of corn roots following a forage radish cover crop was not different than colonization following no cover crop. We speculate that the reason for this lack of a negative effect is that toxic levels of isothiocyanates (ITCs) did not occur throughout the bulk of the soil when forage radish residues were decomposing. Future studies should investigate actual ITC concentrations in both bulk soil and rhizosphere soil during decomposition of forage radish cover crops to confirm this speculation. Furthermore, little is known about the concentrations of ITCs that are toxic to AMF, so this also needs to be known to confirm our speculation. Further research on the actual ITC concentration in soils during forage radish decomposition would be useful information particularly for using forage radish as a biofumigant crop to control plant diseases.

We learned that a rye cover crop mixed with forage radish did not increase subsequent AMF colonization of corn roots, while a pure stand of rye did. Forage radish may somehow interfere with a companion plant's ability to maintain the AMF symbiosis. Further exploration of this is particularly important as increasing numbers of farmers are planting mixed cover crop stands that include forage radish.

The results of this study are somewhat restricted, however, because all experimental sites had optimal levels of P. The benefits to crop nutrition contributed by AMF decline in high P soils. Therefore, if a study similar to ours were conducted in a low P soils, the results may differ. Future research in low P soils is certainly warranted.

Another important finding of this study is that forage radish plants cause increased soil test P concentrations within 3 cm of the tap root holes that remain in spring after decomposition of the roots. A relatively simple calculation showed that approximately 60% of a field can be within 3 cm of a radish root hole when forage radish is planted and becomes well established using recommended seeding rates. Only slight increases in soil test P occurred in the surface 2.5 cm of soil when soil samples were collected at least 5 cm away from radish holes, though. Understanding how this small scale variability in soil test P influences crop nutrition and P losses in runoff and erosion is necessary to design appropriate soil sampling guidelines for both environmental and soil fertility management.

Finally, both forage radish and rye cover crops shoots were able to accumulate large quantities of P. Increasing P removal from excessively high P fields by harvesting cover crops for use as forage or for bioenergy production should be considered. Given the ability of forage radish to take up large quantities of P quickly in the fall, a double cover crop system of forage radish planted in late August and harvested in mid-October, followed by a late planting of rye, could be successful in Maryland.

Appendix A: Sample SAS Codes

Codes used to analyze corn root and shoot data

```
Proc mixed data=corn convf;
class rep cover year field;
model arcsinamfcol= cover|year|field;
random  field field*rep field*rep*cover;
*repeated /group=field*year; *used to assign heterogenous variances to
treatments groups when necessary;
lsmeans cover*field*year / pdiff;
by stage;
run;
```

Codes used to analyze soil test P around radish root holes

```
Proc mixed data=radhole convf;
class rep cover position depth width;
model P= cover*position ; *covb;
random rep;
repeated position/ subject=rep*cover type = SP(EXPA)(depth width);
lsmeans position*cover/ pdiff = control ('No Cover' '16')
adjust=dunnet;
run;
```

Codes used to analyze soil test P in bulk soil

```
proc mixed data = working;
class rep plot cover depth year;
model P= cover|depth|year ;
random rep rep*cover;
repeated depth / subject=rep*cover*year type = CS;
lsmeans cover*year  year*depth /pdiff;
run;
```

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