

# Forage radish and cereal rye cover crop effects on mycorrhizal fungus colonization of maize roots

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**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 fungus colonization of and P acquisition by a subsequent maize (*Zea mays* L.) silage crop. Cover crop treatments included forage radish, rye, a mix of forage radish and rye, and no cover crop. Mycorrhizal fungus colonization of maize 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 maize roots compared to no cover crop. These findings suggest that forage radish cover crops do not have a negative effect on AMF colonization of subsequent crops.

**Keywords** Arbuscular mycorrhizal fungi · Forage radish · Cereal rye · Phosphorus · Isothiocyanate

## Abbreviations

AMF arbuscular mycorrhizal fungi  
P phosphorus  
ITC isothiocyanate

## Introduction

Arbuscular mycorrhizal fungi (AMF) form a symbiotic relationship with the roots of most agricultural crops and aid the roots in the acquisition of soil phosphorus (P) (Brundrett 2004). Arbuscular mycorrhizal fungi are obligate symbionts and 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 maize (*Zea mays* L.) when the soil is P deficient (Boswell et al. 1998; Deguchi et al. 2007; Gavito and Miller 1998b; Kabir and Koide 2002). In soil with adequate plant available P, increased mycorrhizal fungus colonization has generally not translated into increased yields

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(Galvez et al. 2001; McGonigle and Miller 1993; Sorensen et al. 2005).

Forage radish (*Raphanus sativus* L. var. *long-ispinnatus*) 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 maize silage crops. Forage radish is a member of the *Brassicaceae* family, one of the few plant families whose members do not host AMF (Ocampo et al. 1980; Vierheilig et al. 2000). Plants in the *Brassicaceae* 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, b; Vierheilig et al. 2000; Vierheilig and Ocampo 1990a, b). 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 root extracts of *Brassicaceae* plants *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, 1990b). Schreiner and Koide (1993a, b) observed that living roots of *Brassicaceae* plants in sterilized soil, or root extracts of *Brassicaceae* plants in fresh soil, reduced germination of AMF spores after 5 and 7 days. However, 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 *Brassicaceae* members under field conditions is toxic to AMF. Some studies have shown that compared to an AMF host crop, *Brassicaceae* crops reduce colonization of the subsequent crop during its early growth stages (Gavito and Miller 1998a; Sorensen et al. 2005). Several studies, however, have shown that *Brassicaceae* 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 *Brassicaceae* pre-crops as compared to a bare-fallow treatment (Fontenla et al. 1999), but the study was conducted in a potted

mixture of sand and vermiculite, which may not degrade ITCs as effectively as a natural soil.

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 AMF 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 *Brassicaceae* crop 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 *Brassicaceae* 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°C. Following its death, glucosinolates in the forage radish tissue are converted into ITCs and are released into the soil and atmosphere. Other *Brassicaceae* 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 under low temperatures (Matthiessen and Shackleton 2005), which occur 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 maize 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 fungus 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 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 (*Raphanus sativus* L. var. *longipinnatus*, seed source: Steve Groff Seeds, Holtwood, PA), 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 management history

At CMREC, prior to the start of the experiment a rotation of maize, followed by winter wheat (*Triticum aestivum* L.), and double crop soybeans (*Glycine max* L.) 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

**Table 1** Selected properties of the experimental sites at the University of Maryland Central Maryland Research and Education Center (CMREC) and Beltsville Agricultural Research Center North Farm (BARC-NF) and South Farm (BARC-SF)

	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 <sub>w</sub>	5.9 (0.30) <sup>a</sup>	5.6 (0.08)	6.9 (0.13)
Organic Matter <sup>b</sup> (g 100 g <sup>-1</sup> )	1.1 (0.14)	1.3 (0.18)	1.6 (0.05)
Mehlich 3 P (mg kg <sup>-1</sup> )	88 (5.31)	102 (5.98)	98 (1.72)
Sand <sup>c</sup> (g 100 g <sup>-1</sup> )	78	54	50
Silt (g 100 g <sup>-1</sup> )	17	42	38
Clay (g 100 g <sup>-1</sup> )	5	4	12

<sup>a</sup> Where multiple samples were measured, standard error is listed in parentheses; (n=4)

<sup>b</sup> Soil Organic matter by loss on ignition

<sup>c</sup> Particle size analysis by the hydrometer method

promote cover crop growth on the sandy soil at this site. When the soybeans were mowed, the above ground dry matter contained  $56 \text{ kg N ha}^{-1}$  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. At 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. At BARC-SF, prior to the start of the experiment sweet maize 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. On 30 August 2006 fertilizers were broadcast applied at rates of  $84 \text{ kg ha}^{-1}$  of K as potassium chloride,  $17 \text{ kg ha}^{-1}$  of P as triple super phosphate, and  $62 \text{ kg ha}^{-1}$  of N as urea.

#### Cover crop management

At all sites the cover crop treatments were planted using a no-till drill with 16 cm row spacing. Rye was seeded at a rate of  $135 \text{ kg ha}^{-1}$  and forage radish was seeded at a rate of  $14 \text{ kg ha}^{-1}$ . At CMREC, 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. In 2007 at all sites, prior to planting the second year of cover crop treatments weeds were controlled with glyphosate (N-(phosphonomethyl)glycine) ( $1.85 \text{ L ha}^{-1}$  active ingredient (a.i.)) and  $22 \text{ kg N ha}^{-1}$  as a urea ammonium nitrate (UAN) solution was applied as a starter fertilizer for the cover crops to ensure adequate growth. Cover crops were planted at CMREC on 12 September 2006 and 28 August 2007, and at BARC-NF and BARC-SF on 31 August 2006 and 27 August 2007. Weeds in the no cover crop plots were controlled with glyphosate ( $1.85 \text{ L ha}^{-1}$  a.i.) on

18 September 2007 at CMREC, 3 October 2007 at BARC-NF, and 4 October 2007 at BARC-SF and with paraquat dichloride (1,1'-dimethyl-4,4'-bipyridinium dichloride) ( $0.68 \text{ L ha}^{-1}$  a.i.) on 5 November 2007 at BARC-SF. At all sites the forage radish cover crop was killed naturally when temperatures dropped below  $-4^{\circ}\text{C}$  in January of both years. The rye cover crops were killed when all plots were sprayed at CMREC with paraquat dichloride ( $0.68 \text{ L ha}^{-1}$  a.i.) and 2,4-D (2,4-dichlorophenoxyacetic acid) ( $1.05 \text{ L ha}^{-1}$  a.i.) on 10 April 2007 and 12 April 2008, at BARC-NF with paraquat dichloride ( $0.68 \text{ L ha}^{-1}$  a.i.) on 11 April 2007 and glyphosate ( $1.85 \text{ L ha}^{-1}$  a.i.) and 2,4-D ( $1.05 \text{ L ha}^{-1}$  a.i.) on 16 April 2008, and at BARC-SF with glyphosate ( $1.85 \text{ L ha}^{-1}$  a.i.) on 10 April 2007 and 16 April 2008.

#### Maize management

Maize (Pioneer 38B84, glyphosate tolerant) was planted with a no-till planter with 75 cm row spacing at a rate of  $74,000$  seeds  $\text{ha}^{-1}$  at CMREC on 23 April 2007 and 16 April 2008 and at BARC-NF and BARC-SF on 24 April 2007 and 7 May 2008. At CMREC,  $22 \text{ kg N ha}^{-1}$  as a UAN solution was applied at the soil surface in a band 5 cm to the side of the seed furrow as a starter fertilizer at planting. At BARC-NF and BARC-SF,  $22 \text{ kg N ha}^{-1}$  as granular ammonium nitrate was applied in a band 5 cm to the side of the seed furrow and 5 cm deep as a starter fertilizer at planting. Maize was sidedressed with  $112 \text{ kg N ha}^{-1}$  as a UAN solution at CMREC knifed in to a depth of 10 cm between every second maize row on 6 June 2007 and 6 June 2008 and at BARC-NF and BARC-SF dribbled on the soil surface between rows on 7 June 2007 and 10 June 2008. Weeds were controlled in the maize crop at CMREC with glyphosate ( $1.85 \text{ L ha}^{-1}$  a.i.) on 7 May 2007 and glyphosate ( $1.85 \text{ L ha}^{-1}$  a.i.), atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) ( $0.42 \text{ L ha}^{-1}$  a.i.), s-metolachlor (acetamide, 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy<sup>-1</sup>-methylethyl)-, (S)) ( $0.43 \text{ L ha}^{-1}$  a.i.), and mesotrione (2-[4-(methylsulfonyl)-2-nitrobenzoyl]<sup>-1</sup>,3-cyclohexanedione) ( $0.05 \text{ L ha}^{-1}$  a.i.) on 16 May 2008, at BARC-NF with glyphosate ( $1.85 \text{ L ha}^{-1}$  a.i.) on 9 May 2007 and 18 June 2008, and at BARC-SF with glyphosate ( $1.85 \text{ L ha}^{-1}$  a.i.) on 7 June 2007 and glyphosate ( $2.77 \text{ L ha}^{-1}$  a.i.), atrazine ( $1.49 \text{ L ha}^{-1}$  a.i.), and s-

metolachlor (1.61 L ha<sup>-1</sup> a.i.) on 10 June 2008. Maize was harvested as silage on 16 August 2007 and 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. Cover crops were sampled at CMREC and BARC-SF on 15 November 2006, 1 April 2007, 9 November 2007, and 7 March 2008 and at BARC-NF on 29 November 2006, 7 April 2007, 20 November 2007 and 11 April 2008. Cover crop samples were obtained by removing plant parts from two 0.25 m<sup>2</sup> 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°C for a minimum of 7 days. Samples were weighed then ground and sieved to <2 mm particle size and stored in sealed polyethylene vials.

#### Maize root and shoot sampling

Maize shoots were sampled for analysis of dry matter accumulation and tissue phosphorus concentration at the V4 (4th leaf), V8 (8th leaf) and R1 (silking) growth stages. At CMREC, sampling dates occurred in 2007 on 15 May (V4), 13 June (V8), and 9 July (R1) and in 2008 on 19 May (V4), 10 June (V8), and 30 June (R1). At BARC-NF, sampling dates occurred in 2007 on 18 May (V4), 25 June (V8), and 11 July (R1) and in 2008 on 6 June (V4) and 25 June (V8). Samples were not collected in the R1 stage at BARC-NF in 2008. At BARC-SF, sampling dates occurred in 2007 on 15 May (V4), 11 June (V8), and 6 July (R1) and in 2008 on 9 June (V4), 20 June (V8), and 14 July (R1). The field plots at BARC-NF and BARC-SF contained 4 maize rows and plant samples were removed from the center two rows. At CMREC field plots contained 8 maize rows and plant samples were taken from the third and sixth row of each plot, except in the rye/radish mixed plots. In each of these plots, two maize rows which were positioned between a radish and a rye cover crop row were selected for sampling. At each sampling date, four 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 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 maize 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.

Maize 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°C 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 maize 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°C for a maximum of 24 h before being washed to remove all soil particles. Root samples were then immediately dried in a forced draft oven at 60°C and stored until analysis for mycorrhizal fungus colonization.

#### Silage yield sampling

Samples to determine silage yield were collected from the same maize 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°C for a minimum of 7 days to determine the moisture content, which was used to calculate dry matter weight of the silage maize.

#### 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°C for 5 h. 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 fungus colonization analysis

The dried maize root systems were separated into fine roots (< 1 mm diameter) and coarse roots with only the fine roots analyzed for mycorrhizal fungus 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 h and stained in 0.05% Trypan Blue (w/v) stain at room temperature for 6 h. Stained roots were placed on an 8 cm×8 cm Petri dish and viewed under a dissecting microscope (SMZ-2 T, Nikon, Tokyo, Japan) at 60X magnification. Mycorrhizal fungus colonization was assessed as a percentage of root length colonized using the grid-line intersect method (Giovannetti and Mosse 1980). Total mycorrhizal fungus colonization was recorded, which included colonization by hyphal, arbuscular, and vesicular fungal structures.

#### Statistical analysis

To detect treatment effects, 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 maize 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.

An ANCOVA model in the Mixed procedure of SAS was used to determine if there were significant linear relationships between AMF colonization of V4 maize roots and V4 shoot P concentration, V8 shoot P concentration or silage dry matter yield and if those relationships interacted with sites and years. The full ANCOVA model included the terms V4 AMF colonization, V4 AMF colonization X Site, V4 AMF colonization X Site X Year, Site, Year, and Site X Year. Non-significant terms were removed from the full model to create a reduced model through an iterative process in which the highest order non-significant ( $P > 0.05$ ) interaction with V4 AMF colonization was removed in each iteration. If an interaction with V4 AMF colonization was significant, all lower order interactions that were contained within the significant interaction were retained in the model. When a significant interaction occurred between V4 AMF colonization and site or year, slopes of the linear relationships were compared using estimate statements in SAS.

## Results

### Cover crop growth

Dry matter production of cover crops varied among sites and between years (Table 2). At CMREC in both years, cover crops produced less dry matter than at the other sites. At BARC-NF and BARC-SF rye and forage radish achieved shoot dry matter production over 4,000 kg ha<sup>-1</sup> in all years and exceeded

**Table 2** Dry matter production of cover crops at each site year. Cover crop treatments were planted at three sites in August/September of 2006 and 2007. Values indicate the means (SE);  $n=4$

Site	Cover	Dry matter	
		2006 Planting	2007 Planting
		kg ha <sup>-1</sup>	
CMREC	Radish <sup>a</sup>	1,306 (260)	2,629 (260)
	Rye <sup>b</sup>	1,711 (260)	1,546 (260)
	Radish/Rye Mixed		
	Radish in Mix	771 (260)	1,821 (272)
	Rye in Mix	869 (260)	763 (272)
	Total Mixed	1,640 (260)	2,584 (272)
	BARC-NF		
BARC-NF	Radish	5,583 (569)	4,642 (569)
	Rye	4,177 (569)	7,062 (569)
BARC-SF	Radish	4,282 (404)	4,026 (404)
	Rye	7,345 (404)	4,117 (404)

<sup>a</sup>Radish shoots were sampled in the late fall

<sup>b</sup>Rye shoots were sampled in spring

7,000 kg ha<sup>-1</sup> of rye at BARC-NF in 2008 and BARC-SF in 2007.

#### Mycorrhizal fungus colonization

There was a cover crop effect on the percent of root length colonized by AMF at the V4 stage of maize growth (Table 3). There was also a significant year by site interaction on AMF colonization at both V4 and V8 growth stages. There was no cover crop effect on AMF colonization of maize roots at the V8 growth stage (Table 3).

At CMREC, the rye cover crop caused greater V4 maize root colonization than any of the other cover crop treatments in both 2007 and 2008 (Table 4). There was no difference in V4 maize 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 maize root colonization between the cover crop treatments in 2007 but in 2008 the rye cover crop caused greater V4 maize root colonization than no cover crop. At BARC-SF in 2007, the rye cover crop caused greater V4 maize root colonization than the forage radish

cover crop. At BARC-SF in 2008 there were no significant differences in V4 maize root colonization between the cover crop treatments. Maize 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, maize 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 4). At the V8 stage, maize 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.

#### Maize shoot P concentration

There were significant interactions between cover crop and site upon shoot P concentration at the V4 stage and between cover crop and year at the V8 stage. At the R1 (silk) stage, cover crop had a significant effect on maize shoot P concentration (Table 3). There was also a significant year by site interaction at all growth stages.

At CMREC, V4 maize 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 4). At CMREC in 2008, V4 maize 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 maize shoot P concentration in either year.

In 2007, there were no significant differences between cover crop treatments in V8 maize shoot P concentrations at any of the sites (Table 4). In 2008, V8 maize 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 maize 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 maize shoot P concentration.

At CMREC, R1 stage maize shoots following a rye cover crop had a greater P concentration than after all other cover crop treatments in 2008, and a greater P concentration than no cover and forage radish treatments in 2007 (Table 4). Across all sites and maize

**Table 3** Significance of cover crop treatment effects on AMF colonization of maize roots, maize shoot P concentration, and maize dry matter production at various growth stages. Following termination of the cover crop treatments, maize was planted in April/May of 2007 and 2008 at three sites. To maximize power, all site years were pooled into a single ANOVA

Source of variation	DF <sup>a</sup>	Root colonization		Shoot P concentration			Dry matter production			
		V4	V8	V4	V8	R1 (Silk)	V4	V8	R1 (Silk)	Yield
Cover	21	<0.001 <sup>b</sup>	0.984	0.001	0.002	<0.001	0.008	0.010	<0.001	0.086
Year	27	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.167	<0.001
Cover x Year	27	0.239	0.891	0.187	0.002	0.452	0.005	0.126	0.705	0.043
Site	0	.	.	.	.	.	.	.	.	.
Cover x Site	21	0.088	0.716	0.003	0.099	0.628	0.002	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	0.005
Cover x Year x Site	27	0.673	0.809	0.262	0.180	0.818	0.019	0.060	0.046	0.002

<sup>a</sup>DF error degrees of freedom

<sup>b</sup>Values indicate the probability of a greater F-value ( $\alpha=0.05$ )

**Table 4** Cover crop effects on AMF colonization of maize roots and maize shoot P concentration, at various growth stages. Following termination of the cover crop treatments, maize was planted in April/May of 2007 and 2008 at three sites

Site	Cover crop	Maize root colonization			Maize shoot P concentration		
		V4	%	V8	V4	V8 g kg <sup>-1</sup>	R1 (Silk)
CMREC 2007							
	Mix	31a <sup>a</sup>		37 <sup>b</sup>	3.87a	4.49a	2.62ab
	No Cover	32a		37	3.75a	4.18a	2.45a
	Radish	33a		38	3.81a	4.04a	2.41a
	Rye	46b		37	5.27b	4.54a	2.79b
BARC-NF 2007							
	No Cover	14a		51	2.49a	2.85a	2.52a
	Radish	14a		56	1.84a	3.02a	2.43a
	Rye	18a		49	2.07a	3.35a	2.65a
BARC-SF 2007							
	No Cover	22ab		36	2.28a	3.66a	2.43a
	Radish	18a		32	1.82a	4.04a	2.43a
	Rye	27b		33	1.79a	3.93a	2.70a
CMREC 2008							
	Mix	62a		61	6.43b	5.22b	3.21a
	No Cover	61a		59	6.57b	5.01ab	3.18a
	Radish	66a		63	5.19a	4.47a	3.18a
	Rye	77b		61	8.67c	6.30c	3.64b
BARC-NF 2008							
	No Cover	32a		48	3.05a	3.20a	n.d. <sup>c</sup>
	Radish	40ab		45	2.76a	2.82a	n.d.
	Rye	44b		45	3.72a	4.10b	n.d.
BARC-SF 2008							
	No Cover	70a		54	5.45a	4.62a	3.00a
	Radish	72a		53	5.62a	4.46a	3.00a
	Rye	72a		57	5.59a	4.67a	3.26a

<sup>a</sup> Within site and year, means in a column followed by different letters are significantly different (F-protected LSD,  $P<0.05$ )

<sup>b</sup> Mean comparisons were not made for V8 maize root colonization because the ANOVA did not indicate a significant cover crop treatment effect

<sup>c</sup> nd Not determined

growth stages, maize 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 at BARC-NF.

#### Maize dry matter

There were significant interactions between cover crop, site, and year for maize dry matter production at the V4 and R1 growth stages and at silage harvest (Table 3). At the V8 growth stage, there was a significant interaction between cover crop and site for maize dry matter production.

At CMREC in 2007, forage radish increased maize 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 5). There were no other significant differences in maize growth at CMREC in 2007. In 2008 at CMREC, all cover crop treatments increased maize 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 maize dry matter compared to no cover crop.

At BARC-NF in 2007, both forage radish and rye increased maize dry matter at the V4, V8, and R1 stages compared to no cover crop (Table 5). Both forage radish and rye also increased silage yield compared to no cover crop. At BARC-NF in 2008, compared to no cover crop, radish increased maize dry matter at the V4 stage and rye increased maize 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 maize dry matter at the V4 stage, but increased silage yield compared to no cover crop (Table 5). In 2008 at BARC-SF, the rye cover crop decreased maize 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 maize dry matter at all growth stages.

#### Relationship between AMF colonization, maize shoot P, and maize silage yield

The relationship between AMF colonization and maize shoot P concentration at the V4 stage was different between sites (Table 6). At all sites, a significant positive linear relationship existed, but

**Table 5** Cover crop effects on maize dry matter production at various growth stages. Following termination of the cover crop treatments, maize was planted in April/May of 2007 and 2008 at three sites.

Site	Cover	Stage			
		V4	V8	R1 (Silk) kg ha <sup>-1</sup>	Silage
CMREC 2007					
	Mix	20.4a <sup>a</sup>	683a	5,817ab	9,543a
	No Cover	16.3a	715a	5,094a	9,763a
	Radish	18.8a	1,045b	6,254b	10,198a
	Rye	18.7a	745ab	5,483ab	9,241a
BARC-NF 2007					
	No Cover	9.0a	1,123a	2,708a	7,225a
	Radish	17.7b	1,854b	4,290b	9,870b
	Rye	20.2b	1,895b	4,233b	10,959b
BARC-SF 2007					
	No Cover	17.6b	749a	5,205a	12,157a
	Radish	16.9ab	791a	5,980a	12,755ab
	Rye	13.2a	654a	5,602a	14,798b
CMREC 2008					
	Mix	32.4ab	807b	4,249b	13,911ab
	No Cover	26.6a	400a	2,949a	11,825a
	Radish	34.0b	868b	4,090b	14,721b
	Rye	28.1ab	759b	4,163b	14,008ab
BARC-NF 2008					
	No Cover	16.8a	502a	n.d. <sup>b</sup>	8,548a
	Radish	22.5b	617ab	n.d.	10,164a
	Rye	21.4ab	706b	n.d.	11,213a
BARC-SF 2008					
	No Cover	122b	937b	7,128b	16,046b
	Radish	113b	848b	7,789b	17,020b
	Rye	36.9a	501a	5,698a	9,759a

<sup>a</sup> Within site and year, means in a column followed by different letters are significantly different (F-protected LSD,  $P < 0.05$ )

<sup>b</sup> nd Not determined

the slope of the relationship was greater at CMREC than at BARC-NF and BARC-SF (Fig. 1). A significant positive relationship between AMF colonization of maize roots at the V4 stage and maize shoot P concentration at the V8 stage also existed, and was similar among sites and years (Table 6). There was no correlation between AMF colonization and silage yield (Table 6).

**Table 6** The significance of linear relationships between AMF colonization of V4 maize roots and shoot P concentration at the V4 and V8 stage and silage dry matter yield were tested in an ANCOVA model with site and year as categorical explanatory variables and AMF colonization as a continuous explanatory variable

Source of variation	Response variable		
	P <sup>a</sup> V4	P V8	Silage Yield
AMF V4 <sup>b</sup>	<0.001 <sup>c</sup>	0.042	n/s <sup>d</sup>
AMF V4 X Year	n/s	n/s	n/s
AMF V4 X Site	<0.001	n/s	n/s
AMF V4 X Site X Year	n/s	n/s	n/s
Year	n/s	0.932	0.002
Site	n/s	0.008	0.002
Year X Site	n/s	0.037	0.024

<sup>a</sup> P = Maize shoot P concentration at each stage

<sup>b</sup> AMF V4 = Proportion of V4 maize root length colonized by AMF

<sup>c</sup> Values indicate the probability of a greater F-value ( $\alpha=0.05$ )

<sup>d</sup> n/s = Non-significant terms of the model were removed iteratively

## Discussion

### Mycorrhizal fungus colonization

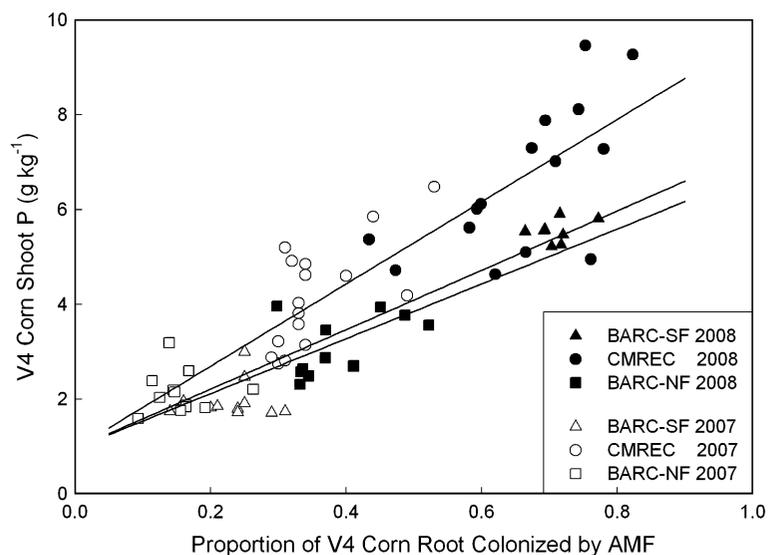
Forage radish cover crops never caused decreased levels of AMF colonization of the subsequent maize

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 so any volatile ITCs released during decomposition would diffuse 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). The cold soil temperatures during the period of time when ITCs were released after the radish was killed by freezing air temperatures may have decreased the toxicity of ITCs to AMF as well (Matthiessen and Shackleton 2005). Finally, ITCs

**Fig. 1** Relationship between AMF colonization of maize roots and maize shoot P concentration at the V4 stage within each site and pooled across years. The relationship is significant at all sites ( $P<0.01$ ), and the slope of the relationship at CMREC is greater than at BARC-NF and BARC-SF ( $P<0.01$ )



may have a degree of toxicity to fungal hyphae, but may only be fungistatic to resting or dormant structures such as spores (Schreiner and Koide 1993a, b).

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 *Brassicaceae* 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 *Brassicaceae* crops reduces subsequent AMF colonization, but these have been in comparison to a rotation of an AMF host crop rather than a fallow year (Gavito and Miller 1998a; Sorensen et al. 2005).

An AMF non-host crop grown during the winter, such as forage radish, should have less of an effect on subsequent AMF colonization than an AMF non-host crop grown in the summer because the metabolic needs of AMF are relatively minimal in the winter. An undisturbed network of AMF extraradical hyphae can retain its full colonization potential through a period of cold temperatures even in the absence of a host plant (Lekberg and Koide 2008; McGonigle and Miller 1999). During a warm temperature fallow period, however, the colonization potential of extraradical hyphae will decline (Lekberg and Koide 2008) because AMF respiration in warm temperatures will deplete carbon reserves in the absence of a host.

At CMREC, a rye cover crop resulted in increased AMF colonization of the next maize 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, 2002; Karasawa et al. 2001; Sorensen et al. 2005). At BARC-NF, mycorrhizal fungus colonization was greater following rye only when compared to no cover crop. At BARC-SF there were no differences in AMF colonization of maize 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 fungus 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 cutting the

mycelium of AMF into fragments, 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 fungus 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 fungus 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.

The no-till soil management during the experiment may have influenced the effect of the cover crops on AMF colonization of the maize roots. Boswell et al. (1998) found that no-till enhanced AMF colonization of a maize crop after a winter wheat cover crop but not after a winter fallow. In our study, the no-till management may have enhanced the effect of the rye cover crop compared to the forage radish cover crop and no cover crop. The no-till management may have also limited the quantity and distribution of ITCs released by forage radish, thus minimizing any negative effect of forage radish on AMF. Because of the possible interactions with tillage, the results of this study should not be applied to conditions where tillage occurs.

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 root of a *Brassicaceae* plant 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 *Brassicaceae* plant, 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 maize roots was greater in the second year of the study than in the first year 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 fungus colonization than the cooler and drier conditions in the spring of 2007 (Fig. 2). The favorable weather in spring 2008 may have improved early maize growth, allowed the maize plants to allocate more resources to supporting AMF colonization, and may have stimulated more rapid AMF spore germination and hyphal growth.

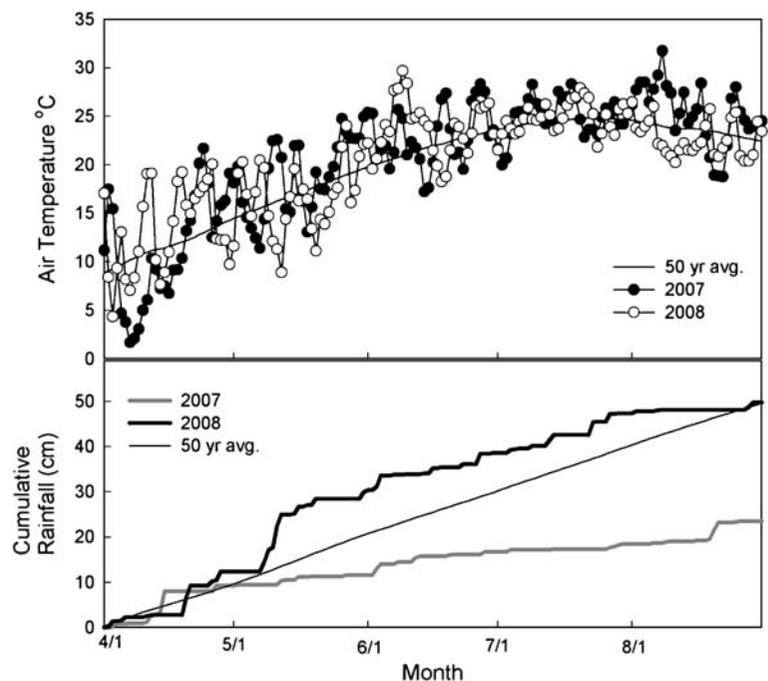
#### Maize growth

In 5 of the 6 site years, at least one of the cover crop treatments increased maize dry matter production at one or more of the growth stages measured. The most notable effect on maize growth was at BARC-NF in 2007, where rye and forage radish cover crop treat-

ments both resulted in increased maize 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 had below average rainfall (Fig. 2), increased maize 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). Increased maize yield following forage radish at CMREC in 2008 but not in 2007 may have been because of the greater forage radish dry matter production, and thus greater nutrient release upon decomposition, in 2008 as compared to 2007.

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

**Fig. 2** Air temperature and rainfall in Beltsville, MD from April through August during 2007 and 2008



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 2) 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 maize growth.

At BARC-SF in 2008, the rye cover crop decreased maize dry matter throughout the entire season. Maize 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 maize 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 maize 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 maize 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 maize growth at the V4 stage compared to forage radish and no cover crop, although maize 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 maize by decreasing soil temperatures and increasing soil water content.

#### Relationship between AMF colonization, maize shoot P, and maize silage yield

This study found a positive linear relationship between AMF colonization and shoot P concentrations. At CMREC, the slope of the relationship between AMF colonization and shoot P concentration of V4 maize may have been greater than at the other sites because of a more extensive extraradical AMF hyphal network at CMREC owing to the longer history of no-till management. A more extensive hyphal network in the soil could allow the AMF to acquire P from a greater volume of soil and thus deliver greater

quantities of P to the maize plant for a given percentage of root colonization. McGonigle et al. (1990) found that reducing the extent of an intact extraradical hyphal network by cutting the soil into cubes 4 cm and smaller reduced the P concentration of maize shoots but did not reduce the percentage of root colonization. There was no correlation between AMF colonization and maize silage yield, probably because soil test P levels were in the optimum range. Other studies conducted in soil with optimum P levels have also found no correlation between AMF colonization and yield (Galvez et al. 2001, 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 maize crop despite the presumed release of ITCs during its decomposition. The lack of a negative effect might be accounted for by the no-till treatment 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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