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Cover crop species affect mycorrhizae-mediated nutrient uptake and pest resistance in maize

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Abstract

Arbuscular mycorrhizal fungi (AMF) can increase plant nutrient uptake and chemical defense production, both of which can improve plants' ability to resist insect herbivory. Cover crops—non-commercial species planted in between cash crops in a crop rotation—can naturally alter both soil nutrients and AMF. We tested whether different cover crop species alter AMF colonization, plant nutrient status and plant–insect interactions in a subsequent maize crop. Cover crop species were either non-mycorrhizal, non-leguminous (canola, forage radish), mycorrhizal non-leguminous (cereal rye, oats), mycorrhizal leguminous (clover, pea) or absent (fallow). We measured the cascading consequences of cover crop treatment on maize root AMF colonization, maize growth and performance of an herbivorous insect (European corn borer) feeding on the maize. Maize AMF colonization was greater in plots previously planted with mycorrhizal (rye, oats) than non-mycorrhizal (canola, radish) cover crops or no cover crop (fallow). AMF colonization was linked to increased plant phosphorous and nitrogen, and maize growth increased with low plant N:P. Induced jasmonic acid pathway plant defenses increased with increasing maize growth and AMF colonization. European corn borer survivorship decreased with lower plant N:P, and insect development rate decreased with increased induced plant defenses. Our data describe a cascade in which cover crop species selection can increase or decrease mycorrhizal colonization of subsequent maize crop roots, which in turn impacts phosphorus uptake and may affect herbivory resistance in the maize. These results suggest that farmers could select cover crop species to manage nutrient uptake and pest resistance, in order to amend or limit fertilizer and pesticide use.

Introduction

According to recent projections, crop production will need to increase by 25–70% within the next 33 years in order to meet global food demands (Hunter *et al.*, 2017). Sustainable intensification—namely, increasing crop yields from the existing farmlands, while simultaneously reducing the environmental impacts of agriculture, is encouraged as a means to meet these food demands (Baulcombe *et al.*, 2009; Garnett *et al.*, 2013). Reduction of pesticide and fertilizer applications are key to reducing negative agricultural impacts on the environment (Baulcombe *et al.*, 2009); however, in order to reduce these inputs, alternative strategies are needed to effectively improve crop utilization of soil nutrients and natural crop resistance to pests.

Arbuscular mycorrhizal fungi (AMF) will play a critical role in sustainable intensification because they form symbiotic associations with the roots of >80% of plant species (Willis *et al.*, 2013), with potential impacts on both crop nutrient use and pest resistance. It has been well-documented that AMF can increase plant absorption of nutrients, particularly phosphorus (P) (Jeffries *et al.*, 2003; Javaid, 2009; Willis *et al.*, 2013). More recent research has shown that AMF colonization can also increase secondary compound production and defense gene upregulation in crop plants. Root colonization by AMF can increase 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one production and defense against fungal infections in maize seedlings (Song *et al.*, 2011), and AMF colonization can also upregulate induced defense production—that is, a defense produced by a plant, in response to herbivory, that reduces the herbivore's performance and/or increases plant fitness (Karban and Myers, 1989). Specifically, AMF increase crop plant production of chemicals along the jasmonic acid (JA) pathway when plants are damaged (Pozo and Azcón-Aguilar, 2007; Cameron *et al.*, 2013). This can inhibit survivorship and growth of phytophagous insect larvae that feed on crop plants (Pozo and Azcón-Aguilar, 2007; Shivaji *et al.*, 2010; Chuang *et al.*, 2014).

Arbuscular mycorrhizae are present in most soil, but their abundance and ability to colonize cash crop roots is reduced by common farming practices such as tillage and post-harvest

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fallow periods (Kabir, 2005). One farming practice that can increase AMF is cover cropping; planting a noncommercial species in the time period between cash crops, when soil would otherwise be exposed (Finney and Kaye, 2016). Cover crops can significantly increase AMF colonization in the subsequent cash crop, so long as the cover crop species itself forms mycorrhizal associations (Kabir and Koide, 2002; White and Weil, 2010). It is unknown whether cover crops can sufficiently boost AMF colonization, which in turn boost induced plant defenses, in the subsequent cash crop so that it significantly affects pests that feed on those plants.

The impact that cover crops have on soil nutrients can also affect plant growth and defense production, but these impacts vary among common cover crop species. Some cover crops fix nitrogen (N) and are mycorrhizal (legumes), others are mycorrhizal but do not fix N (e.g., grasses), and still others are non-mycorrhizal and do not fix N (e.g., brassicas). Cover crops also provide N via decay of residue biomass after termination (Ranells and Wagger, 1996), though the amount of N provisioned is dependent upon the residue C:N ratio, which varies by cover crop species (Ranells and Wagger, 1996; Teasdale and Abdul-Baki, 1998; Finney *et al.*, 2016) and growth stage of the cover crop (Ranells and Wagger, 1992; Clark *et al.*, 1997). Greater N provided by the cover crop residue, in turn, may improve pest performance and reduce production of secondary metabolites by the fertilized plant (Chen *et al.*, 2010).

While the current state of knowledge suggests that cover crop species selection may be a management choice with cascading impacts on soil nutrients, mycorrhizal colonization and cash crop nutrient uptake and pest resistance, this multitrophic level cascade is difficult to trace and its impact has not been measured in the field. There is a need for field evaluation precisely because of potential conflicting interactions among plant nutrition, plant defense production and herbivore response to AMF colonization in plants. There is evidence in the literature that plant defense can be either positively or negatively correlated with plant growth, depending on the plant species (Viola *et al.*, 2010). There is also species-dependent response of insects to AMF-colonized plants, depending on the type of herbivore, the part of the plant it feeds on, and its degree of host specificity (Koricheva *et al.*, 2009). Neither of these examples precludes the possibility of cover-crop mediated effects on crop defense and pest performance; rather, such variability among plant and insect species signals the need for these multitrophic interactions to be investigated under field conditions.

Here we present a field experiment conducted on developing maize plants grown in 28 organic research plots previously planted with seven different cover crop treatments (Table 1). We conducted this experiment to test the hypotheses that (1) mycorrhizal cover crop species (grasses and legumes) would produce greater AMF colonization in a subsequent maize crop than non-mycorrhizal cover crop treatments (fallow and brassicas), (2) soil nutrients and AMF colonization, as influenced by cover crop species, both affect nutrient uptake in maize plants, (3) plant nutrition and AMF colonization affect constitutive defense (plant defense compounds produced in the absence of damage, infection or feeding; Wittstock and Gershenzon, 2002) and induced defense responses in maize plants and (4) herbivorous insect survivorship and growth are affected by the nutrition and induced defense production of maize plants. These interconnected hypotheses represent a cascade of cover crop influences on maize nutrition and herbivore resistance that could enable farmers to select cover crop species to better manage soil fertility and pests.

Materials and methods

Study site description

We collected data at the Cover Crop Cocktails research site at Rock Springs Agricultural Research Station near Pennsylvania Furnace, PA USA. Detailed descriptions of the crop rotations and fertilization protocols are described in Murrell *et al.* (2017). An aerial map of the field site and arrangement of treatments can be found in Supplement 1. To describe the aspects of the system most relevant to this study, 12 adjacent 0.84 ha blocks of land were organically farmed in a wheat (*Triticum aestivum* L.)–maize (*Zea mays* L.)–soybean (*Glycine max* L Merr.) rotation beginning in 2012 in a fully phased design (i.e., four blocks each of wheat, maize and soybean are grown in any given year). After wheat was harvested in July 2014, the post-wheat blocks were tilled and then divided into twelve 0.07 ha plots which were planted with one of 12 treatments: no cover crop (fallow), one of six cover crop monocultures or one of five mixtures of the cover crop species from the monocultures. The order of cover crop treatments within each block was pre-randomized. For this study we collected data only from the fallow plots and from the six cover crop monocultures: canola (*Brassica napus* L.), forage radish (*Raphanus sativus* L.), Austrian winter pea (*Pisum sativum* L. ssp. *sativum* var. *arvense*), red clover (*Trifolium pratense* L.), oats (*Avena sativa* L.) and cereal rye (*Secale cereale* L.) (Table 1). In total we collected from 28 plots, four plots of each cover crop treatment listed and four fallow plots.

Cumulative monthly precipitation at this field site in the study year, 2015, was 40 mm in May, 173 mm in June and 155 mm in July. Mean ± S.D. precipitation for this region, based on climate data from 2004 to 2014, is 98 ± 42 mm in May, 99 ± 41 mm in June and 105 ± 44 mm in July (NOAA NCDC, 2018). Mean daily temperature in 2015 was 18.4°C in May, 19.9°C in June and 21.8°C in July. Mean ± S.D. daily temperatures per month for this region between 2004 and 2014 were 15.9 ± 1.8°C for May, 20.6 ± 1.0°C for June and 22.7 ± 1.5°C for July (NOAA NCDC, 2018). Collectively summarized, in the year of our field study May was drier and warmer than the historical mean; June and July were wetter than the historical mean, but had typical mean daily temperatures.

Mycorrhizal colonization and nutrient assessments

Data on the spring biomass and C:N ratio of each cover crop are listed in Table 1. Cover crops in these plots were terminated by flail mowing on May 4, 2015. Ten 20 cm × 2.5 cm soil cores were collected from each plot on May 6 and compiled. A subsample of each composite sample was tested for soil % P by the Agricultural Analytical Services Laboratory of The Pennsylvania State University (University Park, PA) using Mehlich 3 inductively coupled plasma analysis (Wolf and Beegle, 2011). Dairy manure was applied on May 11 at 47 Mg ha^{-1} to ¾ of each plot, while the remaining ¼ was left as an unfertilized strip. All plots were moldboard plowed and then disked on May 11–12 after manure application. On May 26th the plots were cultimulched; Masters Choice® MC4050 variety maize was then planted on May 28.

On June 24–25, 2015, ten additional 20 cm × 1.8 cm soil cores were collected within the non-fertilized strips of each plot. These

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Table 1. The seven cover crop treatments used in this study, their mycorrhizal properties, and their growth and nutritional properties in Fall 2014–Spring 2015 within the treatment plots used in this study

Cover crop treatment	Species	Family	Mycorrhizal?	Season of peak biomass	C:N of cover crop at peak biomass	Peak cover crop above-ground biomass (kg/ha ± SE)
Canola	*B. napus* L.	Brassicaceae	No	Spring and Fall equivalent	17.9[a]	1734.2 ± 210.3[a]
Forage radish	*R. sativus* L.	Brassicaceae	No	Fall	14.3	1861.3 ± 423.0
Cereal rye	*S. cereale* L.	Poaceae	Yes	Spring	31.2	2194.1 ± 407.7
Oats	*A. sativa* L.	Poaceae	Yes	Fall	30.4	3413.5 ± 375.3
Austrian winter pea	*P. sativum* L.	Fabaceae	Yes	Fall	11.5	2881.4 ± 351.1
Red clover	*T. pratense* L.	Fabaceae	Yes	Spring	11.3	1365.1 ± 162.7
Fallow (control)	–	–	–		–	0.0 ± 0.0

[a]Fall canola: C:N = 17.3, biomass = 1826.6 ± 276.4. Spring canola: C:N = 18.5, biomass = 1641.8 ± 352.5.

cores were compiled by plot; a 10 g subsample of soil was then taken from each composite soil sample and analyzed for extractable soil N (NH_4^+ plus NO_3^-) using KCL extraction, filtration and calorimetric analysis (White *et al.*, 2017). Maize seedlings were collected at V2–V4 stage in order to determine mycorrhizal colonization of plant roots and above-ground plant tissue nutrient composition. These plants were about 25 days post-emergence, one of the earliest growth stages at which significant mycorrhiza-nutrient correlations have been shown to occur (Kabir and Koide, 2000). Four groups of seedlings (three seedlings per group, combined to ensure sufficient roots and plant tissues for testing) were collected from the 3rd and 5th row from the plot edge, at least 3 m from the border of each plot with the adjacent cover crop plot, in the section of each plot that had not been fertilized with manure. We recorded the development stage and measured the height of each plant from the base of the stem to the whorl notch; mean development stage and mean height was later calculated per replicate.

Roots were trimmed from the plants, rinsed under tap water to remove all soil, and stored by replicate in 50% ethanol in glass 20 mL scintillation vials. To assess AMF colonization, preserved roots were cut into 1-cm segments, cleared in 10% potassium hydroxide solution, and boiled in a solution of vinegar and 5% Sheaffer black ink in order to dye the mycorrhizae (Vierheilig *et al.*, 1998). After dyeing, 15 root sections were randomly selected from each sample, and 100–160 root intersections scanned with a compound microscope at 100× magnification (modified from McGonigle *et al.*, 1990). The number of intersections colonized with arbuscules and/or vesicles was recorded. These data were then used to calculate the proportion of root intersections colonized by AMF.

Maize shoots were bagged by group and dried for five days at 60°C. The dried shoots were ground to 2 mm, and part of this ground tissue was digested using the Kjeldahl digestion method (Koch and McMeekin, 1924). The digested solution was then analyzed for P using the ascorbic acid method (Watanabe and Olsen, 1965). The remaining ground tissue sample from each plant was tested for total N content by dry combustion using a CHNS analyzer (EA 1110, CE Instruments, Italy).

Larval performance assay

On July 9, 2015, four undamaged maize plants of V5 development stage were randomly selected in each of the cover crop treatment plots. Each plant was measured for height, then a leaf tissue sample of approximately 100 mg was collected from the 4th mature leaf of the plant (the oldest mature leaf being the 1st leaf), placed in a 1.5 mL Eppendorf tube, and immediately frozen in liquid nitrogen. Each plant was then infested with 95–105 *Z*-strain European corn borer (*Ostrinia nubilalis* Hübner, hereafter abbreviated as 'ECB') eggs, and covered with a bag of insect mesh which was cable-tied to the base of the maize stem. The larvae were allowed to hatch and feed on the plant for 10 days. This allowed us to assess the length of time necessary for ECBs to develop from egg to approximately 2nd instar, the development time at which they feed on the exterior of the plant (Labatte *et al.*, 1991) and are most susceptible to predation and mortality from abiotic factors (Coll and Bottrell, 1992).

After 9 days of larval feeding, a small hole was cut in each bag to collect a second approximately 100 mg leaf tissue sample from the same leaf that was sampled prior to larval infestation. In the event this leaf was destroyed, the sample was taken from a leaf adjacent to this leaf. This second leaf sample was immediately frozen in liquid nitrogen. The following day each plant was harvested at the stem base and frozen at 0°C. Each frozen plant was measured for final plant height, and all ECB larvae were removed from the plant and counted. The instar of each larva was determined by measuring the width of the prothoracic shield (Cook *et al.*, 2003). Mean larval instar (D) was then calculated for each plant using the following equation (Murrell and Cullen, 2014):

$$D = \frac{[N_{1\text{st instar}} + (N_{2\text{nd instar}} \times 2) + (N_{3\text{rd instar}} \times 3) + (N_{4\text{th instar}} \times 4) + (N_{5\text{th instar}} \times 5)]}{N_{\text{Total ECBs}}}$$

where higher D indicates a greater mean development stage of ECB larvae for a given plant (Murrell and Cullen, 2014). In the context of this study, lower D equates to lower caterpillar growth rate, which increases the likelihood of larval mortality (Coll and Bottrell, 1992).

Assessment of constitutive and induced plant defenses

Plant tissues that were collected pre- and post-herbivore infestation as described above were ground in liquid nitrogen using GenoGrinder 2000 (SPEX sample prep, USA). To 100 mg of ground leaf tissue, Trizol reagent (Life Technologies, USA) was added and RNA was extracted using the manufacturer's protocol. One microgram of total RNA was used to generate cDNA using Oligo dT and high capacity cDNA kit (Life Technologies,

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USA). Quantitative real time polymerase chain reaction (PCR) was performed in 7500 Fast Real Time PCR machine (Applied Bioscience, USA) for three known caterpillar-induced maize defense genes: lipoxygenase3 (LOX3), maize protease inhibitor (MPI) and ribosome-inactivating protein2 (RIP2) using SYBR green reagent (Roche Biosciences, USA) (Louis *et al.*, 2013). Expression level of actin was used as an endogenous control and relative quantification (RQ) values were calculated by the delta–delta Ct method (Livak and Schmittgen, 2001). Sum of the RQs of LOX3, MPI and RIP2 before and after ECB infestation generated total constitutive defenses and total induced defenses respectively. Primers for all gene expression analyses were the same as used by Louis *et al.* (2013). Samples for three of the plots yielded insufficient RNA for analysis; therefore the sample size for all analyses that include RQs is 25.

Statistical analyses

Since this study investigates multitrophic interactions, we analyzed relationships among manipulated treatments and measured variables using mixed linear model analyses (PROC MIXED, SAS 9.4) (SAS, 2009). Data for all models were checked to ensure normal distribution (PROC UNIVARIATE, SAS 9.4) prior to being included in the models. All transformations performed to fit data to meet normality are noted in the individual analyses.

Mean soil N, soil P and % of maize seedling root intersections colonized by AMF were calculated for each plot. Each of these parameters was then analyzed by cover crop treatment using mixed linear model analyses with the plot as replicate and block included as a random effect. Soil N was ln-transformed to meet the assumption of normality. For models in which the cover crop treatment was significant, we performed follow-up multiple pairwise comparisons of least square means with a Tukey adjustment (PROC MIXED, SAS 9.4). Effects of % AMF colonization, soil P and their interaction on plant P across plots was also analyzed with mixed linear models, with the plot as replicate and block as a random effect. Effects of % AMF, soil N and their interaction on plant N were analyzed in the same way.

For the maize plants infested with ECB larvae, we calculated mean plant growth (final height – height at infestation) by plot, then used a mixed linear model to analyzed effects of mean N:P ratios and mean % AMF colonization of the seedlings previously collected from each plot on plant growth, with block as a random effect. Plant growth was square-root transformed to ensure normal distribution and a best fit [lower Akaike's information criterion (AICc)] for the mixed linear model.

RQs for each gene, constitutive and induced, were ln-transformed to ensure normal distribution of data and best model fit. Constitutive RQs were analyzed with mixed linear models to test effects of plant growth by plot, % AMF, and the growth × AMF interaction on each gene, with block included as a random effect. Induced RQs were analyzed with mixed linear models testing effects of plant growth by plot, % AMF, number of surviving larvae on each plant, and all two-way interactions of these variables on each gene, with block included as a random effect. The 3-way interaction in this model was not significant and therefore was removed from the final model.

Since plants had been fed upon for 10 days, we predicted that all genes along the JA pathway were likely induced. Therefore, we summed the RQs of all the genes for both constitutive and induced expression to quantify total defenses in the JA pathway. These summed RQs were ln-transformed and tested using the same analyses described previously for the individual gene RQs.

We used mixed linear model to test effect of summed constitutive RQs, summed induced RQs, and plant N:P ratios on the mean number of surviving larvae per plant, by plot (ln-transformed), with block included as a random effect. Effects of constitutive RQs, induced RQs, plant N:P, and mean surviving larvae on mean larval instar were tested in the same way. For both analyses, two- and three-way interactions were not significant and were removed from the final models.

Results

Cover crops significantly altered extractable soil N, with red clover plots containing higher soil N than cereal rye (Table 2). Cover crops also significantly altered the percentage of maize root intersections colonized by AMF, with colonization in oats and cereal rye plots being significantly higher than colonization in radish plots. Extractable soil P was not correlated with cover crop treatment (Table 2).

Phosphorus content in maize seedlings was significantly positively correlated with both soil % P and % AMF colonization of plant roots (Table 3). There was also a significant interaction effect between soil % P and % AMF such that the effect of % AMF on seedling P decreased as soil % P increased (Table 3, Fig. 1A). Nitrogen content of seedlings also significantly increased as soil % N increased, but % AMF had no significant effect on plant N (Table 3, Fig. 1B). The N:P ratio of maize plants by plot was negatively correlated with V5 plant growth over 10 days (Δheight) (mixed linear model $F_{1,19} = 4.96$, $P = 0.0382$).

Maize defenses induced by experimental herbivory were synergistically correlated with maize growth and % AMF colonization of seedlings within the same plots, such that treatment plots with both high plant growth and high seedling AMF colonization had plants with greater expression of induced defense genes (Table 4, Fig. 2A). There was also a significant interaction effect of % AMF and number of surviving larvae on induced maize defenses such that plots with low seedling AMF had plants with a strong positive induced defense response to larval density, but this response became less predictable as % AMF increased (Table 4, Fig. 2B). In contrast to these interactive effects, the main effects of maize growth or the mean number of surviving larvae per plot (Table 4) did not significantly correlate with induced defense. Constitutive gene expressions of LOX3, MPI, RIP2 and all three defense genes combined were not significantly correlated with plant growth, seedling % AMF colonization, or the interaction between these variables (Table 4). Response of individual constitutive and induced gene expressions can be found in Supplement 2.

The number of surviving larvae decreased as plant N:P decreased, but was not significantly correlated with constitutive or induced plant defenses (Table 3). Mean larval development stage did not correlate with constitutive defense expression, plant N:P, or the mean number of larvae per plant, but was negatively correlated with plant induced defense expression (Table 5, Fig. 3).

Discussion

This study demonstrates that cover crop species differ in their abilities to increase soil nutrients and AMF colonization of young maize plants. The soil legacy created by cover crop

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Table 2. Mixed linear models of cover crop effect on soil N, soil P, and % AMF colonization of maize roots

Model parameter	Soil N (mg/kg)			Soil P (mg/kg)			% AMF Colonization		
	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Cover crop	6,18	3.04	0.0313	6,18	1.06	0.4195	6,18	4.76	0.0045
Estimates									
Fallow		14.84	AB		49.00	A		59.73	AB
Canola		12.68	AB		32.25	A		60.19	AB
Forage radish		15.46	AB		29.50	A		51.09	B
Oats		11.00	AB		38.50	A		75.32	A
Cereal rye		9.49	B		43.00	A		76.68	A
Austrian winter pea		24.46	AB		40.25	A		71.11	A
red clover		27.62	A		42.50	A		66.88	AB
Variance components									
Block		0			240.20			0	
Residual Error		0.21			166.30			73.30	

Model parameter estimates are shown for the cover crop treatments with Tukey-adjusted pairwise comparison results. Estimates that share letters do not significantly differ.

Table 3. Mixed linear model analyses for corn plant leaf phosphorus and nitrogen, as affected by their respective soil nutrients and percent of root intersections colonized by AMF

	Plant % phosphorus			Plant % nitrogen		
Model parameter	df	*F*	*P*	df	*F*	*P*
Soil P	1,21	10.80	0.0035	–	–	–
Soil N	–	–	–	1,22	9.31	0.0059
% AMF Colonization	1,21	21.80	0.0001	1,22	11.33	0.0030
Soil P × % AMF Colonization	1,21	7.47	0.0125	–	–	–

The interaction effect between soil nitrogen and AMF colonization was not significant for plant nitrogen, and therefore was excluded from the final model.

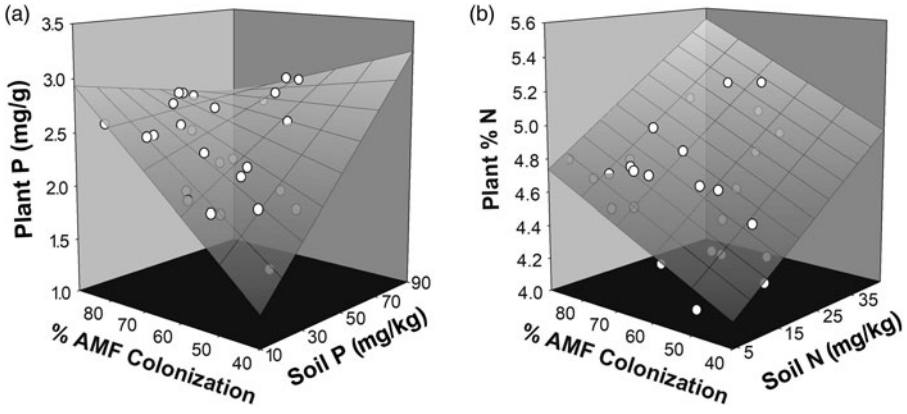

Fig. 1. Relationships between extractable soil nutrient content and % AMF colonization of corn roots on (A) phosphorus content (B) nitrogen content of corn leaf tissue at the V2–V4 stage of development by plot ($n = 28$). Points represent raw data, while the plane represents the relationship between variables as predicted by mixed linear model analyses. Plant P model (AICc = −126.9): $y = -0.04984 + 4.6792$(Soil % P) + 0.3857 (% AMF Colonization) − 5.6402(Soil % P × % AMF Colonization) + 0[block variance] + 0.000437[residual variance]. Plant N model (AICc = 0.9): $y = 3.6948 + 1.1987$(Soil % N) + 1.1796(% AMF Colonization) + 0.005673[block variance] + 0.04488[residual variance].

selection is subsequently correlated with plant nutrition, plant defense gene expression and development of pest insects that feed on the maize. Taken together, these results strongly suggest that the higher AMF colonization increased plant vigor both in terms of induced plant defenses and plant growth. This counters the prevalent narrative that resource partitioning limits plant's ability to grow and defend against herbivores at the same time (Huot *et al.*, 2014).

It is notable that insect survivorship and insect development were correlated with different plant properties. Larval survivorship was significantly associated with high N:P, which in turn was negatively correlated with plant height. This means that short plants with more limited P content actually supported a greater number of larvae, and subsequently were exposed to more damage. Maize in the radish cover crop treatment was particularly low in root AMF colonization and plant P. Subsequently

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Table 4. Mixed linear model analyses of effects of change in plant height (square root-transformed) percent of root intersections colonized by AMF, and the interaction on constitutive defenses, and effects of change in plant height, AMF colonization, number of surviving larvae (ln-transformed), and all two-way interaction on induced plant defenses

	Total constitutive defenses			Total induced defenses		
Model parameter	df	*F*	*P*	df	*F*	*P*
ΔPlant height				1,15	3.90	0.0671
% AMF colonization				1,15	1.65	0.2178
# Larvae	–	–	–	1,15	0.52	0.4802
ΔPlant height × % AMF colonization				1,15	5.78	0.0296*
ΔPlant height × # Larvae	–	–	–	1,15	2.26	0.1534
# Larvae × % AMF colonization	–	–	–	1,15	4.68	0.0471*

*$P < 0.05$.

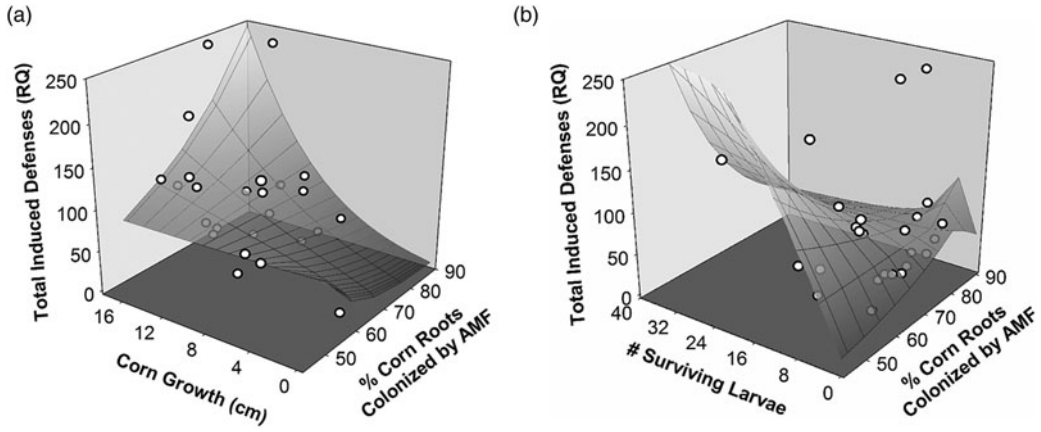

Fig. 2. Relationships between (A) plant growth and % AMF colonization of corn roots, and (B) Larval density and % AMF colonization phosphorus, on RQ of all induced plant defense genes tested (LOX3, MPI and RIP2) by plot ($n = 25$). Points represent raw data, while the plane represents the relationship between variables as predicted by mixed linear model analyses. Induced plant RQ model (AICc = 54.0): $y = 8.3005 - 2.3251$(Plant Growth) $- 0.0572$(% AMF Colonization) $+ 0.7088$(# Larvae) $+ 0.0313$(Plant Growth × % AMF Colonization) $- 0.0262$(% AMF Colonization × # Larvae) $+ 0.4263$(Plant Growth × # Larvae) $+ 0$[block variance] $+ 0.1788$ [residual variance].

Table 5. Mixed linear model analyses for total constitutive defenses, total induced defenses, plant nutrients on *O. nubilalis* survivorship, and these three factors plus larval density on mean larval instar by plant

	# Surviving larvae			Mean larval instar		
Model parameter	df	*F*	*P*	df	*F*	*P*
Total constitutive	1,18	2.43	0.1362	1,17	0.02	0.891
Total induced	1,18	3.49	0.0781	1,17	5.66	0.0294*
Plant N:P	1,18	10.43	0.0046**	1,17	0	0.9465
# Surviving larvae	–	–	–	1,17	1.93	0.1832

*$P < 0.05$, **$P < 0.01$.

these plants supported greater larval survivorship, with a mean ± SE of 14.40 ± 2.59 larvae per plant that was well above the grand mean of 6.74 larvae per plant across all plants in the study.

Larval development was not correlated with plant N:P, but was negatively associated with induced plant defenses, which were highest both in plants with low AMF colonization and high larval density (e.g., radish, canola treatments) and in plants with high AMF colonization and low larval density (e.g., oats and cereal rye treatments). Taking both survivorship and development into account, our data suggest that grass cover crops may be most effective at providing pest resistance to a subsequent maize crop, as these grasses produced the highest AMF colonization in maize roots. The maize plants in these treatments subsequently had lower larval survivorship, and high induced defense expression even with lower larval densities.

The negative correlation between plant growth and N:P ratio suggests that phosphorus uptake make have a proportionally greater effect on early maize development than nitrogen. This is consistent with previous research that early growth maize is particularly sensitive to P availability, and requires relatively greater amounts of P for rapid shoot and leaf development (Bittman

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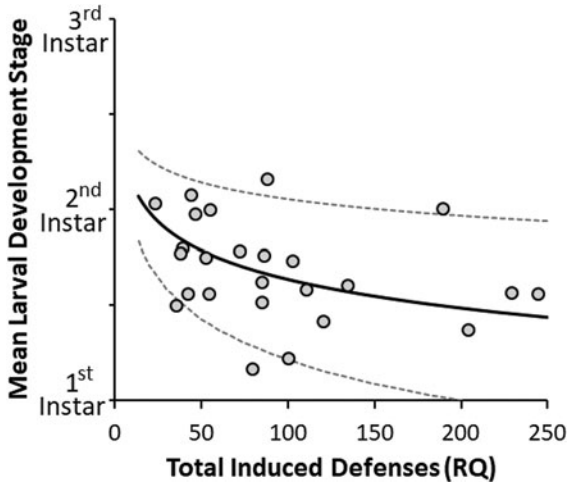

Fig. 3. Negative correlation of mean European corn borer larval development stage and summed qRT-PCR RQ of induced plant defenses among plots ($n = 25$). Points represent raw data by experimental plot, the solid line represents the linear relationship as predicted by mixed linear model analysis and the dashed lines represent the 95% confidence intervals predicted by the mixed linear model analysis. European corn borer larval development model (AICc = 13.4): = 2.2944 + 0.0121(Constitutive Defense RQs) − 0.2170(Induced Defense RQs) + 0.01674(Plant N:P) + 0.1368(# Larvae) + 0[block variance] + 0.0618[residual variance].

et al., 2004). Whether induced plant defenses were increased directly by AMF association with maize roots, indirectly by increased P nutrition in colonized plants, or a combination of the two is unclear. Phosphorus is necessary for transcription when the plant's genes, including plant defense genes, are expressed (Raven, 2013). At the same time, studies have shown that AMF themselves may directly influence both JA and SA pathways as they colonize plant roots (Pozo and Azcón-Aguilar, 2007; Cameron *et al.*, 2013). Further testing is needed to tease apart the indirect *vs* direct effects of AMF and P availability on plant defense production.

It should be noted that the interactions observed in this study have the potential to be affected by other untested variables. Soil phosphorus, for example, varied considerably among field plots, ranging from 10 to 90 mg kg^{-1}. It is possible that other unmeasured soil nutrients were similarly variable among plots and could have affected plant growth. Variations in non-AMF microbes could also have affected plant defense production. For example, the endophytic fungi *Trichoderma* spp. and rhizobacteria *Pseudomonas* spp. can alter induced defense compound production in their plant hosts (Berendsen *et al.*, 2012). A study in which an alfalfa cover crop inoculated with AMF and *Trichoderma* found that AMF colonization, but not *Trichoderma* soil abundance, was increased in a subsequent potato crop (Buysens *et al.*, 2016). A separate study demonstrated that a mustard cover crop, in some soil types, can significantly alter *Pseudomonas* and other bacterial species' abundances in the soil (Collins *et al.*, 2006). Co-variability in non-AMF microbes is therefore possible in these systems, which could lead to AMF being correlated with, but not mechanistically responsible for, the alterations in plant defenses we observed. Future studies more directly manipulating microbial types independently could help to determine which of these, or other, soil microbes may affect plant defenses to pests in the field.

Other variables that could have affected our study include temperature and precipitation. As noted previously, temperatures during our study were typical for the region, while precipitation was lower than typical in the month preceding the experiment, but higher than the previous 11-year mean during the experiment (June–July). A previous study documented no change in AMF colonization of sorghum roots under drought conditions, but an approximate 10% decrease in AMF colonization when soil moisture was high (Deepika and Kothamasi, 2014). However, the soil moisture treatment that achieved this effect was 50–60% water content, in which the soil was continuously submerged in 1 cm of water, a condition that was not observed in our field plots. This is not to say that the correlations we observed between AMF, plant defense and insect response would not be affected by precipitation; however, the amount of precipitation needed to drive these changes would likely have much greater direct agronomic effects on our study system.

Sustainable intensification of agriculture will require in depth knowledge of cascades that link crop rotations, mycorrhizae, nutrients and defense against herbivores. These multi-trophic cascades are difficult to trace and even more difficult to manage. Our research shows that cover crop selection may be a key lever to controlling mycorrhizae-mediated maize P uptake and induced resistance to herbivory. Farmers select cover crops for a range of benefits including N fixation, N retention, erosion prevention, and weed management (CTIC, SARE, and ASTA, 2016). Many of these benefits are incentivized through cost reduction to the farmer (e.g., lower fertilizer costs following legume cover crops) or government policies. Yet, to our knowledge, neither farmers nor policy makers have considered cover crop selection an important choice for improving crop resistance to insect herbivory. If our results translate to other locations and other crops then cover crop selection may enable farmers to manipulate mycorrhizal colonization, P uptake and N availability, which would reduce both fertilizer and pesticide use while maintaining yields.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S1742170519000061.

Data. Data will be uploaded to ResearchGate (https://www.researchgate.net/) upon acceptance of the manuscript.

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