Journal of Applied Ecology

Journal of Applied Ecology 2016, **54,** 1785–1793 doi: 10.1111/1365-2664.12815

Ecological intensification and arbuscular mycorrhizas: a meta-analysis of tillage and cover crop effects

Timothy M. Bowles[1]*, Louise E. Jackson[2], Malina Loeher[2] and Timothy R. Cavagnaro[3]

[1]*Department of Environmental Science, Policy and Management, University of California Berkeley, Berkeley, CA 94720, USA;* [2]*Department of Land, Air and Water Resources, University of California Davis, Davis, CA 95616, USA; and* [3]*The Waite Research Institute and School of Agriculture, Food and Wine, University of Adelaide, Waite Campus, PMB1, Glen Osmond, SA 5064, Australia*

Summary

1. Reliance on ecosystem services instead of synthetic, non-renewable inputs is increasingly seen as key to achieving food security in an environmentally sustainable way. This process, known as ecological intensification, will depend in large part on enhancing below-ground biological interactions that facilitate resource use efficiency. Arbuscular mycorrhizas (AM), associations formed between the roots of most terrestrial plant species and a specialized group of soil fungi, provide valuable ecosystem services, but the full magnitude of these services may not be fully realized under conventional intensively managed annual agricultural systems.

2. Here, we use meta-analysis to assess how reducing soil disturbance and periods without roots in agricultural systems affect the formation of AM and the diversity and community composition of arbuscular mycorrhizal fungi (AMF). We compiled data from 54 field studies across five continents that measured effects of tillage and/or cover cropping on AMF colonization and/or communities and assessed effects of management and environmental factors on these responses.

3. Less intensive tillage and winter cover cropping similarly increased AMF colonization of summer annual cash crop roots by $\sim 30\%$. The key variables influencing the change in AMF colonization were the type of cover crop or the type of alternative tillage, suggesting that farmers can optimize combinations of tillage and cover crops that most enhance AM formation, particularly with no-till systems and legume cover crops.

4. Richness of AMF taxa increased by 11% in low-intensity vs. conventional tillage regimes. Several studies showed changes in diversity and community composition of AMF with cover cropping, but these responses were not consistent.

5. *Synthesis and applications*. This meta-analysis indicates that less intensive tillage and cover cropping are both viable strategies for enhancing root colonization from indigenous arbuscular mycorrhizal fungi (AMF) across a wide range of soil types and cash crop species, and possibly also shifting AMF community structure, which could in turn increase biologically based resource use in agricultural systems.

Key-words: arbuscular mycorrhizal fungi, below-ground biological interactions, cover crops, ecological intensification, ecosystem services, intensive agriculture, meta-analysis, soil biodiversity, tillage

Introduction

Steady increases in crop yields since the green revolution have come at substantial environmental cost (Pingali 2012). Any further increases in yields must not further erode the natural capital upon which agriculture relies, especially in times of environmental change, and must minimize negative effects on ecosystem sustainability. Increasing reliance on supporting and regulating ecosystem services instead of synthetic inputs, that is ecological intensification, is increasingly seen as one way of achieving food security in an environmentally sustainable way (Jackson *et al.* 2012; Bommarco, Kleijn & Potts 2013). Ultimately, this will depend in large part on enhancing

*Correspondence author. E-mail: timothy.bowles@gmail.com

below-ground biological interactions that facilitate resource use efficiency (Jackson *et al*. 2012; Bender, Wagg & van der Heijden 2016).

Crops take up approximately only half of the nutrients in applied inorganic fertilizers, with the remainder at risk of being lost from agroecosystems (Robertson & Vitousek 2009). These losses have widespread and serious consequences for climate change, biodiversity and human health (Erisman *et al*. 2014). Plants have evolved many traits for optimizing nutrient acquisition and utilization (Hodge 2004; York, Nord & Lynch 2013; Lambers, Martinoia & Renton 2015), including the formation of arbuscular mycorrhizas (AM), associations between the roots of most terrestrial plant species and a specialized group of soil fungi (Smith & Read 2008). Arbuscular mycorrhizas also provide valuable ecosystem services beyond nutrient acquisition, such as increasing nutrient retention (Cavagnaro *et al*. 2015), plant drought resistance (Augé, Toler & Saxton 2015) and soil structure formation (Rillig & Mummey 2006). But the full magnitude of these services is often not realized in intensively managed, annual agricultural systems (Gosling *et al*. 2006). High rates of soil disturbance and long periods when roots are not present limit the formation of AMs, due to life-history characteristics of these obligate biotrophs (Smith & Read 2008), which depend on carbon (C) from roots to grow and reproduce. A prior meta-analysis (Lekberg & Koide 2005), including both field and greenhouse studies, showed that reducing soil disturbance and shortening fallow periods does increase colonization of roots by arbuscular mycorrhizal fungi (AMF). But inconsistent results from field studies included in that analysis and many more recent ones (e.g. Gavito & Miller 1998; Duan *et al*. 2010; White & Weil 2010; Higo *et al*. 2014) point to a need to assess how the wide variety of conditions [e.g. soil type, crop species, soil phosphorus (P) status] across these studies influence AM responses.

The composition and diversity of AMF communities is increasingly recognized as an important factor in how plants respond to colonization and potential benefits they receive (Verbruggen & Kiers 2010). Similar to plants, AMF species have different traits that make them functionally distinct (Aguilar-Trigueros *et al*. 2015). For instance, some taxa provide better disease or drought stress resistance while others better enhance nutrient uptake and reduce leaching (Marulanda, Azcón & Ruiz-Lozano 2003; Maherali & Klironomos 2007; Köhl, Lukasiewicz & van der Heijden 2016). Ideally, agricultural management would support a functionally diverse AMF species mixture to increase the multi-functionality of the symbiosis. But intensively managed agricultural systems impose strong filters that limit the community assemblage of AMF species to those that can persist in the face of high rates of disturbance, long fallow periods and often monocultures of plant hosts (Verbruggen & Kiers 2010). Often this selects for ruderal species that invest heavily in reproduction and less in nutrient scavenging or transfer to hosts (Oehl *et al*. 2003; Verbruggen & Kiers 2010; Chagnon *et al*. 2013). By changing disturbance regimes and temporal resource availability, low-intensity tillage and cover cropping would be expected to change AMF community composition and potentially enhance diversity if more niche space is created, for example for slower-growing species (Oehl *et al*. 2009). Conversely, since AMF sometimes prefer specific plant hosts (Johnson *et al*. 2004), AMF communities measured on the same cash crop may not differ to a great extent in spite of differences in agronomic management.

A number of field studies have examined changes in AMF communities in response to less intensive management, so that meta-analysis is now possible for assessing how such management could optimize this below-ground interaction considered central to ecological intensification. The focus here is on the impacts of two management interventions that reduce soil disturbance and periods without roots – the use of low-intensity tillage regimes and cover crops – and their impacts on AM colonization and the AMF community. We evaluated 278 comparisons from 54 field studies published between 1990 and 2015 (see Methods), spanning five continents. Studies on cover cropping encompassed a range of cover crop groups, cash crops and sampling times, and studies on tillage included different tillage types, cash crops and soil texture.

Materials and methods

LITERATURE SEARCH AND DATA COLLECTION

We searched the literature in 2015 using ISI Web of Knowledge (available online). Two separate searches were conducted for assessing effects of cover cropping or alternative tillage on AMF colonization rates on cash crop roots or on the AMF community. Although AMF colonization of roots is not necessarily indicative of AM functionality, for example benefits for plant nutrient uptake or productivity, it is the most widely measured attribute of AM and the best indicator available. For cover cropping, the search terms were 'mycorr*' AND 'cover crop*', which resulted in 108 articles in March 2015. For alternative tillage, the search terms were mycorr* AND ('conservation till*' OR 'no-till*' OR 'reduced till*'), which resulted in 239 articles in October 2015. We screened these articles to meet our selection criteria: (i) a field trial comparing (a) bare winter fallow (i.e. unplanted) vs. cover crop(s), *or* (b) multiple types of tillage, including a comparison between a 'conventional' type (usually a mouldboard plough, i.e. soil inversion) and an alternative (e.g. no-till, chisel till); and (ii) data on AMF colonization rates (i.e. per cent root length colonized) on roots of the subsequent annual cash crop. We also examined studies that analysed AMF community composition from soil or root samples (by spore morphological identification or genetic analysis) following incorporation of the cover crop and/or tillage. Only studies with imposed, replicated treatments at one or more sites were included. We expanded our search by checking the reference lists of studies that met our selection criteria.

Multiple comparisons within a single study (e.g. comparing different cover crop species vs. a single winter fallow control) were considered distinct within-study observations to assess the effect of moderator variables. In total, there were 17 papers comprising 93 comparisons for cover cropping and 30 papers comprising 131 comparisons for alternative tillage (Appendix S1, Supporting Information). For effects on the AMF community, there were 15 papers comparing alternative vs. conventional tillage comprising 25 comparisons for AMF abundance, 15 for AMF richness and 13 for AMF diversity (Appendix S1). The focus was on species richness (i.e. the total number of species or taxa present, based on spore taxonomy or genetics) and the Shannon Index as a metric of diversity. Since only five papers reported on AMF community composition in studies comparing cover crops, these papers were evaluated qualitatively in the discussion. Data were extracted from tables and figures (using WebPlotDigitizer; Rohatgi 2015) in publications meeting the selection criteria.

We examined several factors commonly reported across the studies as moderators: the type of cash crop, the sampling stage for roots and soil available P. Categories for type of cash crop were based on which cash crops were commonly included in the selected studies. Maize *Zea mays* L. was the most common crop in both cover cropping and tillage meta-analyses. Other cash crop categories for cover cropping included the next most common crop, soya bean (*Glycine max* [L.] Merr.) and all other crops. For alternative tillage, other cash crop categories included small grains (e.g. wheat *Triticum aestivum* L. and oats *Avena sativa* L.), legumes (e.g. soya bean and common bean *Phaseolus vulgaris* L.) and all other crops. Sampling stage for roots was based on the phenological stage of the cash crop, including *vegetative*, *flowering* and *maturity*. Where not stated in the journal articles, we estimated these stages by determining the days after planting for each sampling time and matching with crop development timelines from extension resources available close to the study area or in a similar climate. Soil available P (μg P g^{-1} soil) was a continuous variable measured in several ways across the studies, most commonly as Olsen, Mehlich III and Bray, or the measurement method was not reported.

Other explanatory variables were specific to either cover cropping or alternative tillage. For cover cropping, non-AMF hosts included species in the *Brassicaceae* family (e.g. rapeseed *Brassica napus* L. and radish *Raphanus sativus* L.) and buckwheat *Fagopyrum esculentum* Moench, which is considered non-mycorrhizal (Wang & Qiu 2006). Functional groups of cover crops included *graminoids*, *legumes* and *non-legume dicots*. The latter were mostly non-AMF hosts but also included AMF hosts sunflower *Helianthus annuus* L. and dandelion *Taraxacum officinale* F.H.Wigg. Categories of weed control included whether or not weeds were controlled (by herbicides or mechanical control) in the winter fallow treatment and whether or not cover crops were terminated with any form of tillage (e.g. by herbicides or mowing and mulching).

Alternative tillage categories were based on the level of disturbance, including *no-till*, *non-inversion* (e.g. chisel), *shallow inversion* (e.g. shallow disking) or *ridge tillage*. The type of conventional tillage was either *deep inversion* (mouldboard plough, representing the majority of conventional tillage treatments) or *shallow inversion* (same as above). Soil texture was divided into *light* (i.e. high silt and sand content) *loam* and *heavy* (i.e. high clay content) (NRCS 1993). We also noted whether or not a cover crop was present prior to tillage.

DATA ANALYSIS

In our meta-analysis, the log response ratio (ln RR) represents the influence of either cover cropping or alternative tillage on AMF colonization of subsequent cash crop roots:

$$\ln \mathrm{RR} = \ln \bar{X}_t - \ln \bar{X}_c = \ln \frac{\bar{X}_t}{\bar{X}_c}$$

where $\bar{X}_t$ and $\bar{X}_c$ are, respectively, the treatment (cover crop or alternative tillage) and control (winter fallow or conventional tillage) mean calculated for that observation. On the log scale, an effect size of 0 means no difference and a positive value means that cover cropping or alternative tillage has a positive effect on AMF colonization of cash crop roots. The variance of response ratios was calculated according to Hedges, Gurevitch & Curtis (1999) using the standard error and number of replicates reported for each individual study. Where standard errors were not presented or could not be calculated, the authors were contacted to request the missing data. When no information was obtained, standard deviations were imputed based on the ratio of standard deviations and means (of either control or treatment groups) from studies that reported both (Lajeunesse 2013; Ellington *et al.* 2015). The median value of this ratio was used to impute standard deviations for trials that reported only means. A sensitivity analysis assessed the effects of these assumptions and found that almost all results were robust (Appendix S1). We note where particular results were sensitive to the imputed standard deviations.

Response ratios were calculated and analysed using the 'metafor' package (Viechtbauer 2010) in R (R Development Core Team 2015) using a mixed effects approach. A publication-level random effect allowed us to account for non-independence of multiple within-study observations (Mengersen, Gurevitch & Koricheva 2013). A model was first run without any moderator variables to assess the overall heterogeneity, and each moderator was subsequently tested one by one as a sole covariate. A categorical moderator variable was considered to have a significant effect on the change in AMF colonization of cash crop roots when the omnibus test of all model coefficients (i.e. including all levels of a categorical variable) was significant ($P < 0{\cdot}05$) (Viechtbauer 2010). We used funnel plots to confirm there was no evidence of publication bias (Philibert, Loyce & Makowski 2012). All models were fit using restricted maximum likelihood estimation. To facilitate ease of interpretation, mean log response ratios and upper and lower bounds of 95% confidence intervals around the mean were back-transformed ($e^{\ln R}$) and expressed as a per cent change relative to the control.

Results

Field studies spanning five continents (all but Africa and Antarctica; Fig. S1) showed strong positive effects of cover cropping and alternative tillage on AMF colonization of cash crop roots. Cover crops increased colonization of summer cash crop roots by 28·5% (95% CI: 12·1–47·4; Fig. 1) relative to winter fallows. Median colonization rates across all observations were 47 and 37% for cover cropping vs. fallow, respectively (Fig. S2). The change in colonization was greater when the cover crop was an AMF host (30·5 vs. 17·4%), but even non-AMF host cover crops (e.g. radish or rape) significantly increased root colonization (95% CI: 2·2–34·8 for non-AMF hosts and 14·1–49·3 for AMF hosts). Legume cover crops had a greater effect on cash crop root colonization than graminoids or non-legume dicots (Fig. 1). Roots of maize and soya beans, the two most common cash crops in the studies, had similarly higher AMF colonization following a cover crop (95% CI: 16·2–62·8 for maize and 16·5–80·5 for soya beans), but this was not apparent for other cash crops, which encompassed a number of different crop species. The sampling stage of cash crop roots, fallow weed control or prior tillage did not affect the change in colonization of cash crop roots following a cover crop (Fig. 1). Soil available P had a marginally significant ($P = 0{\cdot}08$) negative, but weak, effect on the magnitude of the effect size (Fig. 2).

Across all observations, alternative tillage increased colonization of cash crop roots by 27·0% (95% CI: 14·4–41·0) relative to conventional tillage (Fig. 3). Median colonization rates across all observations were 38 and 29% for low-intensity vs. conventional tillage, respectively (Fig. S2). The strongest influence on the magnitude of change was the type of alternative tillage. No-till increased colonization by 30·3% (95% CI: 17·3–44·8), which was similar to shallow inversion and ridge tillage but higher than the 11·2% (95% CI: −1·5 to 25·6) change for non-inversion tillage. Maize and small grain cash crops had less of a change in root colonization than legumes or other cash crops (e.g. sorghum, flax or cotton). The presence of a prior cover crop affected how AMF colonization responded to alternative tillage, increasing colonization by 41·5% (95% CI: 24·0–61·5) compared to 23·8% (95% CI: 11·7–37·2) when no cover crop was present. All cover crops grown in field studies comparing tillage treatments were AM legumes (e.g. hairy vetch *Vicia villosa* Roth). The sampling stage of roots did not affect the change in colonization. Whereas the overall effect of soil texture was not significant, colonization in heavy (i.e. clayey) soils showed no change from alternative tillage, whereas changes occurred in colonization in light and loam textured soils (Fig. 3). The type of conventional tillage did not affect the change in colonization,

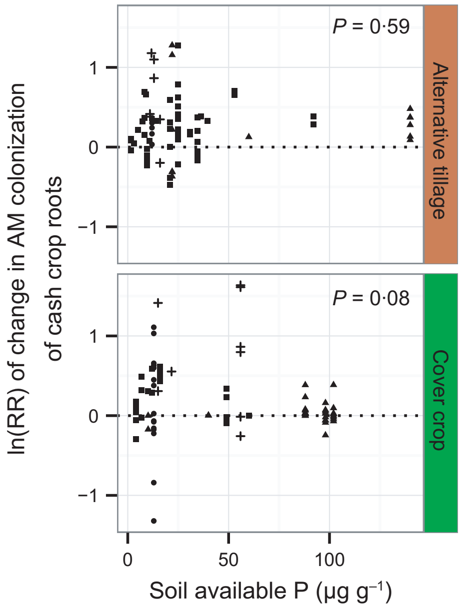

Fig. 2. Meta-analysis results of the change in arbuscular mycorrhizal fungi colonization of cash crop roots as affected by soil available phosphorus (P) levels for field studies on alternative tillage and cover cropping. Symbols are different measurement methods for soil available P. *Circles*: Bray; *triangles*: Mehlich III; *squares*: Olsen; *crosses*: all other methods. ln(RR): log response ratio. The significance of the linear regressions is shown in the upper right, separately for alternative tillage and cover cropping. [Colour figure can be viewed at wileyonlinelibrary.com]

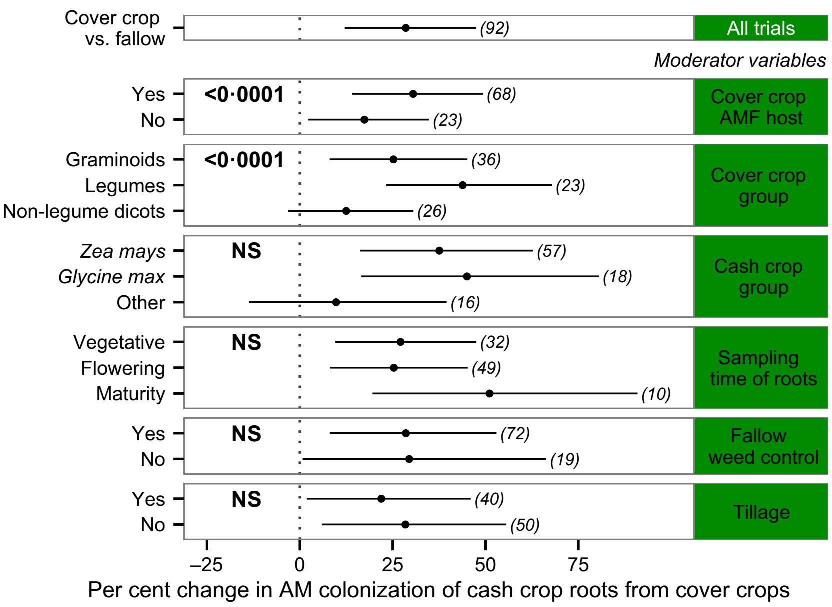

Fig. 1. Meta-analysis results of the change in arbuscular mycorrhizal fungi colonization of cash crop roots in response to fall/winter cover cropping from field experiments in five continents. Error bars represent 95% confidence intervals. Omnibus tests of significance for moderator variables are shown on the left (NS: 'not significant'). The number of observations in each category is shown in parentheses. [Colour figure can be viewed at wileyonlinelibrary.com]

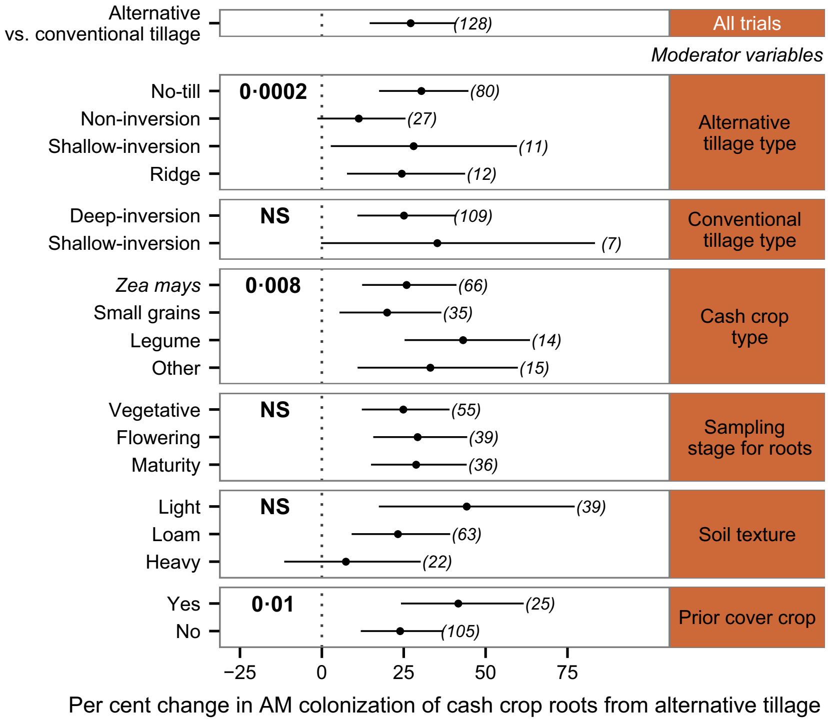

Fig. 3. Meta-analysis results of the change in arbuscular mycorrhizal fungi colonization of cash crop roots in response to alternative tillage from field experiments in five continents. Error bars represent 95% confidence intervals. Omnibus tests of significance for moderator variables are shown on the left (NS: 'not significant'). The number of observations in each category is shown in parentheses. [Colour figure can be viewed at wileyonlinelibrary.com]

although only a small number of trials were shallow inversion (Fig. 3).

Richness of AMF taxa increased by 11·3% (95% CI: 1·0–22·6) in alternative tillage regimes compared to conventional tillage (Table 1). A metric of diversity, the Shannon Index, was not significantly different for AMF taxa in alternative vs. conventional tillage regimes. Within the set of studies reporting effects on AMF community composition or diversity, alternative tillage increased AMF abundance by 60·5%, as measured primarily by spore counts in soil, although the response was highly heterogeneous (95% CI: 15·5–123·0).

Discussion

Although it is often stated that reducing soil disturbance and bare fallows increases AM formation (Gosling *et al.* 2006; de Vries & Bardgett 2012; Schipanski *et al.* 2014), a lack of a systematic analysis of results across field studies have precluded decisive conclusions about the relative efficacy of these interventions, and the key management and soil factors that moderate their effect. The results of this meta-analysis show that across replicated field studies from five continents, less intensive tillage and winter cover cropping similarly increased AM formation in summer annual cash crop roots by ~30%. These results suggest that farmers could optimize combinations of tillage and cover crops that most enhance AM formation, particularly with no-till systems and legume cover crops. But importantly, cover crops increased AM formation similarly whether tillage was used or not, suggesting that the continuity of root associations with cover crops is at least as important for AM formation as decreasing disturbance. This is a significant finding, especially for agricultural systems that may rely more heavily on services provided by AM, for instance organic management (Gosling *et al.* 2006) or low-input systems used by most of the world's farmers (Cardoso & Kuyper 2006). In such systems, tillage is often required for weed control and incorporation of organic matter into the soil (Smukler *et al.* 2008). When a cover crop, especially a legume, is used in these systems, then AM formation in the cash crop apparently can withstand some tillage. Although AMF colonization rates are widely measured, their relationship with actual functions remains unclear (Lekberg & Koide 2005), so future work that uses innovative approaches like non-AM plant mutants (Watts-Williams & Cavagnaro 2015) will be needed to determine decisively how and when these changes are linked to enhancements in ecosystem services like crop productivity.

The 11% increase in richness of AMF taxa in response to alternative tillage suggests that lower intensity soil disturbance creates more niche space in the rhizosphere and root zone that accommodates tillage-sensitive taxa, for example those that rely more on intact root fragments or extraradical mycelia vs. spores for AM formation. Other studies showing that changes in diversity and community composition of AMF are possible (e.g. Ramos-Zapata *et al.* 2012; Higo *et al.* 2013; Säle *et al.* 2015), but not consistent (e.g. Hu *et al.* 2015; Njeru *et al.* 2015), with alternative tillage or cover cropping suggest that determining how to manage AMF community composition will be somewhat site-specific, and tailored to farming goals for productivity and environmental quality.

	Response ratio estimate	95% CI lower bound	95% CI upper bound	Number of observations (*n*)	Number of studies
Abundance	0·4730	0·1441	0·8019	25	9
Richness	0·1072	0·0103	0·2040	15	9
Diversity (Shannon)	0·0256	−0·0673	0·1186	13	7

Table 1. Response ratios and 95% confidence intervals (CI) for arbuscular mycorrhizal fungi abundance, community richness and diversity (the Shannon Index) in response to alternative tillage from field experiments. Response ratios that do not overlap zero are considered significant

IMPACTS OF COVER CROPPING AND ALTERNATIVE TILLAGE ON AMF COLONIZATION

The 28·5% increase in AMF colonization of cash crop roots following a winter cover crop may be a result of increased AMF spore abundance in soil (Lehman *et al.* 2012; Njeru *et al.* 2015). Since AMF are obligate biotrophs, they require C resources from roots to grow and reproduce (Smith & Read 2008), which are not available during a fallow period. Reduced AMF colonization in crops grown after long plant-free periods has been associated with poor crop growth and P and zinc deficiencies (Thompson, Clewett & Fiske 2013). The larger effect of cover cropping on colonization rates reported in a previous meta-analysis (90% increase; Lekberg & Koide 2005) may be due to the inclusion of greenhouse experiments in that meta-analysis, which showed a greater positive response than field experiments (Lekberg & Koide 2005). The stronger response of AMF colonization to legume cover crops compared to graminoids or non-legume dicots likely reflects the high mycorrhizal dependency of typical legume cover crops (e.g. *V. villosa* and *Trifolium* spp.), which could lead to greater spore production and higher levels of colonization in the cash crop (Galvez *et al.* 1995; Njeru *et al.* 2014).

Whereas reductions in AM formation could be expected following a non-AM cover crop species, either as a result of a reduction in AMF populations (similar to a bare fallow) or production of fungal inhibitory compounds like isothiocyanates by *Brassicaceae*, experimental results have been inconsistent (Gavito & Miller 1998; Pellerin *et al.* 2007; White & Weil 2010; Koide & Peoples 2012). In this study, the change in AMF colonization in cash crop roots was indeed greater following cover crops that were AMF hosts compared to non-AMF hosts, but there was still a significant increase in colonization following a non-AM cover crop. This may in part be related to the presence of weeds that are AMF hosts in the non-AM cover crop treatment (Njeru *et al.* 2014) or differences in soil moisture and temperature patterns in cover cropped vs. fallow soils that impact spore viability, for instance. It is also possible that additional organic matter from non-AM cover crops make soil physical properties more conducive to hyphal growth and colonization of subsequent crops (Drew, Murray & Smith 2006).

The 27·0% increase in AMF colonization in alternative tillage regimes compared to conventional tillage reflects the detrimental effects of soil disturbance on AMF hyphal networks (Evans & Miller 1990) and resulting reductions in root colonization (Lekberg & Koide 2005). The stronger response of no-till compared to other forms of alternative tillage was expected since it eliminates belowground disturbance and thus leaves mycelial networks intact, which form an important component of inoculum potential (Evans & Miller 1990; Kabir 2005). In this meta-analysis, the positive interaction between tillage and cover cropping may have been accentuated because all cover crops included in trials evaluating alternative tillage were legumes.

Soil P availability did not strongly affect the response of AMF colonization of cash crop roots to cover cropping or alternative tillage. Using just the most commonly reported test for available P (Olsen; 34% of studies) also showed no relationship with the change in colonization. Nor was a relationship found between soil available P levels in control treatments and the level of AMF colonization, which may be more affected by soil available P than the response ratio (Bolan, Robson & Barrow 1984). High soil P may reduce the plant growth response from AM more than the rate of colonization (Sorensen, Larsen & Jakobsen 2005), but not necessarily (Köhl, Lukasiewicz & van der Heijden 2016).

IMPACTS OF COVER CROPPING AND ALTERNATIVE TILLAGE ON AMF COMMUNITIES

The slight (11%) increase in AMF species richness in response to alternative tillage, but lack of changes in a diversity index (measured by the Shannon Index), suggests that alternative tillage has relatively small effects on AMF habitats, or arrival of additional taxa is slow after a change in soil disturbance. Taxonomic changes can occur in the absence of changes in AMF diversity or richness (e.g. Jansa *et al.* 2002, 2003) in response to types of tillage. For instance, Jansa *et al.* (2003) observed that *Scutellospora* sp. were absent in maize roots from ploughed or chisel tilled plots but present in no-till plots, while several species in the genus formerly known as *Glomus* (Krüger *et al.* 2012) were more prevalent in tilled soils, and *Gigaspora* sp. were present in all treatments, suggesting differing dependences of these genera on an intact hyphal network for survival and root colonization.

The few field-based studies that examined the effect of fall/winter cover cropping on AMF communities show limited changes in response to cover cropping (Table 2). For instance, Ramos-Zapata *et al.* (2012) showed on

Table 2. Effects of fall/winter cover crops on arbuscular mycorrhizal fungi (AMF) species richness, diversity and community composition from field studies

Cover crops	Cash crop	Effect on AMF species richness?	Effect on AMF species diversity?	Effect on AMF community composition?	Study
Vicia villosa Roth, *Brassica juncea* (L.) Coss, Mix	*Solanum lycopersicum* L.	No	No	No	Njeru *et al.* (2015)
Ttriticum aestivum (L.), *Trifolium pretense* L., *Brassica napus* L.	*Glycine max* (L.) Merr	No	No	No	Higo *et al.* (2014)
T. aestivum	*G. max*	Yes, *T. aestivum* > fallow	Yes, *T. aestivum* > fallow	Yes	Higo *et al.* (2013)
T. aestivum, *B. napus*	*G. max*	No	No	Yes	Higo *et al.* (2015)
Mucuna deeringiana (Bort) Merr.	*Zea mays* L.	Yes, *M. deeringiana* > fallow	NA	Yes	Ramos-Zapata *et al.* (2012)

average approximately four more AMF taxa (identified from spores in trap cultures) following a velvetbean *Mucuna deeringiana* [Bort] Merr. cover crop compared to a non-weeded fallow (10·7 vs. 6·3 species) at the end of a 13-year experiment. Specifically, spores from *Acaulospora* and *Rhizophagus* sp. were found only in cover cropped soils. But several other studies (Higo *et al.* 2014, 2015; Njeru *et al.* 2015) did not show any changes in AMF richness or diversity in soil or roots following cover crops in multi-year trials. Fallow treatments tended towards AMF genera with larger spores, perhaps indicating greater viability during long fallows, whereas cover crops tended to support greater abundance of some species in the former genus *Glomus* (Ramos-Zapata *et al.* 2012; Higo *et al.* 2013). While cover crops and associated weeds offer a different host environment and more resources compared to fallows, other management practices (e.g. continued tillage) may constrain changes in AMF community composition.

MANAGEMENT IMPLICATIONS AND CONCLUSIONS

This meta-analysis shows that cover cropping and reducing soil disturbance are strategies that farmers can use to increase AM formation and potentially alter the AMF community across a wide range of soil types and cash crops. Specifically, combining no-till and legume cover cropping would best increase AMF colonization of cash crop roots, highlighting positive interactions across management practices. But cover cropping even appears to counteract some of the negative impacts of soil disturbance on AM formation. System approaches that combine cover cropping and reduced tillage with other AM-promoting practices like crop diversification and organic management (Oehl *et al.* 2004; Verbruggen *et al.* 2010) may offer the most promise for enhancing AM communities, while also increasing soil C storage and nutrient cycling, and reducing nutrient losses and soil erosion (Quemada *et al.* 2013; McDaniel, Tiemann & Grandy 2014; Schipanski *et al.* 2014). Fostering indigenous AMF communities through plant choices and soil management could become an essential component of ecological intensification, which relies on such 'service providing organisms' to support crop productivity while reducing environmental impacts and external inputs (Bender, Wagg & van der Heijden 2016). Future work that links changes in AMF root colonization and functional diversity with specific ecosystem functions would help optimize agricultural systems for both food production and environmental quality.

Acknowledgements

We sincerely thank the authors of all the field studies that contributed to this meta-analysis. Funding to T.M.B. was provided by a U.S. NSF GROW Fellowship and the University of Adelaide. Funding to T.R.C. was supported by the Australian Research Council (FT120100463) and the University of Adelaide. NIFA support through the California Agricultural Experiment Station (CA-D-LAW-2085-H) is acknowledged.

Data accessibility

Data used for the meta-analysis are archived in Dryad Digital Repository http://dx.doi.org/10.5061/dryad.1756f (Bowles *et al.* 2016).

References

Aguilar-Trigueros, C.A., Hempel, S., Powell, J.R. *et al.* (2015) Branching out: towards a trait-based understanding of fungal ecology. *Fungal Biology Reviews*, **29**, 1–8.

Augé, R.M., Toler, H.D. & Saxton, A.M. (2015) Arbuscular mycorrhizal symbiosis alters stomatal conductance of host plants more under drought than under amply watered conditions: a meta-analysis. *Mycorrhiza*, **25**, 13–24.

Bender, S.F., Wagg, C. & van der Heijden, M.G.A. (2016) An underground revolution: biodiversity and soil ecological engineering for agricultural sustainability. *Trends in Ecology & Evolution*, **31**, 1–13.

Bolan, N., Robson, A. & Barrow, N. (1984) Increasing phosphorus supply can increase the infection of plant roots by vesicular-arbuscular mycorrhizal fungi. *Soil Biology and Biochemistry*, **16**, 419–420.

Bommarco, R., Kleijn, D. & Potts, S.G. (2013) Ecological intensification: harnessing ecosystem services for food security. *Trends in Ecology & Evolution*, **28**, 230–238.

Bowles, T.M., Jackson, L.E., Loeher, M. & Cavagnaro, T.R. (2016) Data from: Ecological intensification and arbuscular mycorrhizas: a meta-analysis of tillage and cover crop effects. *Dryad Digital Repository*, http://dx.doi:10.5061/dryad.1756f

Cardoso, I.M. & Kuyper, T.W. (2006) Mycorrhizas and tropical soil fertility. *Agriculture, Ecosystems and Environment*, **116**, 72–84.

Cavagnaro, T.R., Bender, S.F., Asghari, H.R. & van der Heijden, M.G.A. (2015) The role of arbuscular mycorrhizas in reducing soil nutrient loss. *Trends in Plant Science*, **20**, 283–290.

Chagnon, P.L., Bradley, R.L., Maherali, H. & Klironomos, J.N. (2013) A trait-based framework to understand life history of mycorrhizal fungi. *Trends in Plant Science*, **18**, 484–491.

Drew, E.A., Murray, R.S. & Smith, S.E. (2006) Functional diversity of external hyphae of AM fungi: ability to colonise new hosts is influenced by fungal species, distance and soil conditions. *Applied Soil Ecology*, **32**, 350–365.

Duan, T., Shen, Y., Facelli, E., Smith, S.E., Nan, Z., Plant, S. & June, N. (2010) New agricultural practices in the Loess Plateau of China do not reduce colonisation by arbuscular mycorrhizal or root invading fungi and do not carry a yield penalty. *Plant and Soil*, **331**, 265–275.

Ellington, E.H., Bastille-Rousseau, G., Austin, C., Landolt, K.N., Pond, B.A., Rees, E.E., Robar, N. & Murray, D.L. (2015) Using multiple imputation to estimate missing data in meta-regression. *Methods in Ecology and Evolution*, **6**, 153–163.

Erisman, J.W., Galloway, J.N., Seitzinger, S., VBleeker, A., Dise, N., Petrescu, R., Leach, A. & Vries, W. (2014) Consequences of human modification of the global nitrogen cycle. *Philosophical Transactions of the Royal Society B*, **368**, 1–9.

Evans, B.Y.D.G. & Miller, M.H. (1990) The role of the external mycelial network in the effect of soil disturbance upon vesicular-arbuscular mycorrhizal colonization of maize. *New Phytologist*, **114**, 65–71.

Galvez, L., Douds, D.D., Wagoner, P., Longnecker, L.R., Drinkwater, L.E. & Janke, R.R. (1995) An overwintering cover crop increases inoculum of VAM fungi in agricultural soil. *American Journal of Alternative Agriculture*, **10**, 152–156.

Gavito, M.E. & Miller, M.H. (1998) Early phosphorus nutrition, mycorrhizae development, dry matter partitioning and yield of maize. *Plant and Soil*, **199**, 177–186.

Gosling, P., Hodge, A., Goodlass, G. & Bending, G. (2006) Arbuscular mycorrhizal fungi and organic farming. *Agriculture, Ecosystems and Environment*, **113**, 17–35.

Hedges, L.V., Gurevitch, J. & Curtis, P.S. (1999) The meta-analysis of response ratios in experimental ecology. *Ecology*, **80**, 1150–1156.

Higo, M., Isobe, K., Yamaguchi, M., Drijber, R.A., Jeske, E.S. & Ishii, R. (2013) Diversity and vertical distribution of indigenous arbuscular mycorrhizal fungi under two soybean rotational systems. *Biology and Fertility of Soils*, **49**, 1085–1096.

Higo, M., Isobe, K., Drijber, R.A. *et al.* (2014) Impact of a 5-year winter cover crop rotational system on the molecular diversity of arbuscular mycorrhizal fungi colonizing roots of subsequent soybean. *Biology and Fertility of Soils*, **50**, 913–926.

Higo, M., Isobe, K., Kondo, T., Yamaguchi, M., Takeyama, S., Drijber, R.A. & Torigoe, Y. (2015) Temporal variation of the molecular diversity of arbuscular mycorrhizal communities in three different winter cover crop rotational systems. *Biology and Fertility of Soils*, **51**, 21–32.

Hodge, A. (2004) The plastic plant: root responses to heterogeneous supplies of nutrients. *New Phytologist*, **162**, 9–24.

Hu, J., Yang, A., Wang, J., Zhu, A., Dai, J., Wong, M.H. & Lin, X. (2015) Arbuscular mycorrhizal fungal species composition, propagule density, and soil alkaline phosphatase activity in response to continuous and alternate no-tillage in Northern China. *Catena*, **133**, 215–220.

Jackson, L.E., Pulleman, M.M., Brussaard, L. *et al.* (2012) Social-ecological and regional adaptation of agrobiodiversity management across a global set of research regions. *Global Environmental Change*, **22**, 623–639.

Jansa, J., Mozafar, A., Anken, T., Ruh, R., Sanders, I.R. & Frossard, E. (2002) Diversity and structure of AMF communities as affected by tillage in a temperate soil. *Mycorrhiza*, **12**, 225–234.

Jansa, J., Mozafar, A., Kuhn, G. & Anken, T. (2003) Soil tillage affects the community structure of mycorrhizal fungi in maize roots. *Ecological Applications*, **13**, 1164–1176.

Johnson, D., Vandenkoornhuyse, P.J., Leake, J.R., Gilbert, L., Booth, R.E., Grime, J.P., Young, J.P.W., Read, D.J. & Booth, E. (2004) Plant communities affect arbuscular mycorrhizal fungal diversity and community composition in grassland microcosms. *New Phytologist*, **161**, 503–515.

Kabir, Z. (2005) Tillage or no-tillage: impact on mycorrhizae. *Canadian Journal of Plant Science*, **85**, 23–29.

Köhl, L., Lukasiewicz, C.E. & van der Heijden, M.G.A. (2016) Establishment and effectiveness of inoculated arbuscular mycorrhizal fungi in agricultural soils. *Plant, Cell and Environment*, **39**, 136–146.

Koide, R.T. & Peoples, M.S. (2012) On the nature of temporary yield loss in maize following canola. *Plant and Soil*, **360**, 259–269.

Krüger, M., Krüger, C., Walker, C., Stockinger, H. & Schüssler, A. (2012) Phylogenetic reference data for systematics and phylotaxonomy of arbuscular mycorrhizal fungi from phylum to species level. *New Phytologist*, **193**, 970–984.

Lajeunesse, M.J. (2013) Recovering missing or partial data from studies: a survey of conversions and imputations for meta-analysis. *Handbook of Meta-Analysis in Ecology and Evolution* (eds K. Mengersen, J. Gurevitch & J. Koricheva), pp. 195–206. Princeton University Press, Princeton, NJ, USA.

Lambers, H., Martinoia, E. & Renton, M. (2015) Plant adaptations to severely phosphorus-impoverished soils. *Current Opinion in Plant Biology*, **25**, 23–31.

Lehman, R.M., Taheri, W.I., Osborne, S.L., Buyer, J.S. & Douds, D.D. (2012) Fall cover cropping can increase arbuscular mycorrhizae in soils supporting intensive agricultural production. *Applied Soil Ecology*, **61**, 300–304.

Lekberg, Y. & Koide, R.T. (2005) Is plant performance limited by abundance of arbuscular mycorrhizal fungi? A meta-analysis of studies published between 1988 and 2003. *New Phytologist*, **168**, 189–204.

Maherali, H. & Klironomos, J.N. (2007) Influence of phylogeny on fungal community assembly and ecosystem functioning. *Science*, **316**, 1746–1748.

Marulanda, A., Azcón, R. & Ruiz-Lozano, J.M. (2003) Contribution of six arbuscular mycorrhizal fungal isolates to water uptake by *Lactuca sativa* plants under drought stress. *Physiologia Plantarum*, **119**, 526–533.

McDaniel, M., Tiemann, L. & Grandy, A. (2014) Does agricultural crop diversity enhance soil microbial biomass and organic matter dynamics? A meta-analysis. *Ecological Applications*, **24**, 560–570.

Mengersen, K., Gurevitch, J. & Koricheva, J. (2013) *Handbook of Meta-Analysis in Ecology and Evolution*. Princeton University Press, Princeton, NJ, USA.

Njeru, E.M., Avio, L., Sbrana, C., Turrini, A., Bocci, G., Bàrberi, P. & Giovannetti, M. (2014) First evidence for a major cover crop effect on arbuscular mycorrhizal fungi and organic maize growth. *Agronomy for Sustainable Development*, **34**, 841–848.

Njeru, E.M., Avio, L., Bocci, G., Sbrana, C., Turrini, A., Bàrberi, P., Giovannetti, M. & Oehl, F. (2015) Contrasting effects of cover crops on 'hot spot' arbuscular mycorrhizal fungal communities in organic tomato. *Biology and Fertility of Soils*, **51**, 151–166.

NRCS (1993) *Soil Survey Manual*. United States Department of Agriculture, Lincoln, NE, USA.

Oehl, F., Sieverding, E., Ineichen, K., Mäder, P., Boller, T., Wiemken, A. & Ma, P. (2003) Impact of land use intensity on the species diversity of arbuscular mycorrhizal fungi in agroecosystems of central Europe. *Applied and Environmental Microbiology*, **69**, 2816–2824.

Oehl, F., Sieverding, E., Mäder, P., Dubois, D., Ineichen, K., Boller, T. & Wiemken, A. (2004) Impact of long-term conventional and organic farming on the diversity of arbuscular mycorrhizal fungi. *Oecologia*, **138**, 574–583.

Oehl, F., Sieverding, E., Ineichen, K., Mäder, P., Wiemken, A. & Boller, T. (2009) Distinct sporulation dynamics of arbuscular mycorrhizal fungal communities from different agroecosystems in long-term microcosms. *Agriculture, Ecosystems and Environment*, **134**, 257–268.

Pellerin, S., Mollier, A., Morel, C. & Plenchette, C. (2007) Effect of incorporation of *Brassica napus* L. residues in soils on mycorrhizal fungus colonisation of roots and phosphorus uptake by maize (*Zea mays* L.). *European Journal of Agronomy*, **26**, 113–120.

Philibert, A., Loyce, C. & Makowski, D. (2012) Assessment of the quality of meta-analysis in agronomy. *Agriculture, Ecosystems and Environment*, **148**, 72–82.

Pingali, P. (2012) Green revolution: impacts, limits, and the path ahead. *Proceedings of the National Academy of Science*, **109**, 12302–12308.

Quemada, M., Baranski, M., Nobel-de Lange, M.N.J., Vallejo, A. & Cooper, J.M. (2013) Meta-analysis of strategies to control nitrate leaching in irrigated agricultural systems and their effects on crop yield. *Agriculture, Ecosystems and Environment*, **174**, 1–10.

R Development Core Team (2015) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Ramos-Zapata, J.A., Marrufo-Zapata, D., Guadarrama, P., Carrillo-Sánchez, L., Hernández-Cuevas, L. & Caamal-Maldonado, A. (2012)

Impact of weed control on arbuscular mycorrhizal fungi in a tropical agroecosystem: a long-term experiment. *Mycorrhiza*, **22**, 653–661.
Rillig, M.C. & Mummey, D.L. (2006) Mycorrhizas and soil structure. *New Phytologist*, **171**, 41–53.
Robertson, G.P. & Vitousek, P.M. (2009) Nitrogen in agriculture: balancing the cost of an essential resource. *Annual Review of Environment and Resources*, **34**, 97–125.
Rohatgi, A. (2015) WebPlotDigitizer. http://arohatgi.info/WebPlotDigitizer/
Säle, V., Aguilera, P., Laczko, E., Mäder, P., Berner, A., Zihlmann, U., van der Heijden, M.G. & Oehl, F. (2015) Impact of conservation tillage and organic farming on the diversity of arbuscular mycorrhizal fungi. *Soil Biology and Biochemistry*, **84**, 38–52.
Schipanski, M.E., Barbercheck, M., Douglas, M.R. *et al.* (2014) A framework for evaluating ecosystem services provided by cover crops in agroecosystems. *Agricultural Systems*, **125**, 12–22.
Smith, S.E. & Read, D.J. (2008) *Mycorrhizal Symbiosis*, 3rd edn. Academic Press, Cambridge, UK.
Smukler, S.M., Jackson, L.E., Murphree, L., Yokota, R., Koike, S.T. & Smith, R.F. (2008) Transition to large-scale organic vegetable production in the Salinas Valley, California. *Agriculture, Ecosystems and Environment*, **126**, 168–188.
Sorensen, J.N., Larsen, J. & Jakobsen, I. (2005) Mycorrhiza formation and nutrient concentration in leeks (*Allium porrum*) in relation to previous crop and cover crop management on high P soils. *Plant and Soil*, **273**, 101–114.
Thompson, J.P., Clewett, T.G. & Fiske, M.L. (2013) Field inoculation with arbuscular-mycorrhizal fungi overcomes phosphorus and zinc deficiencies of linseed (*Linum usitatissimum*) in a vertisol subject to long-fallow disorder. *Plant and Soil*, **371**, 117–137.
Verbruggen, E. & Kiers, E.T. (2010) Evolutionary ecology of mycorrhizal functional diversity in agricultural systems. *Evolutionary Applications*, **3**, 547–560.
Verbruggen, E., Röling, W.F.M., Gamper, H.A., Kowalchuk, G.A., Verhoef, H.A. & van der Heijden, M.G.A. (2010) Positive effects of organic farming on below-ground mutualists: large-scale comparison of mycorrhizal fungal communities in agricultural soils. *New Phytologist*, **186**, 968–979.
Viechtbauer, W. (2010) Conducting meta-analyses in R with the metafor package. *Journal of Statistical Software*, **36**, 1–48.
de Vries, F.T. & Bardgett, R.D. (2012) Plant–microbial linkages and ecosystem nitrogen retention: lessons for sustainable agriculture. *Frontiers in Ecology and the Environment*, **10**, 425–432.
Wang, B. & Qiu, Y.L. (2006) Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza*, **16**, 299–363.
Watts-Williams, S.J. & Cavagnaro, T.R. (2015) Using mycorrhiza-defective mutant genotypes of non-legume plant species to study the formation and functioning of arbuscular mycorrhiza: a review. *Mycorrhiza*, **25**, 587–597.
White, C.M. & Weil, R.R. (2010) Forage radish and cereal rye cover crop effects on mycorrhizal fungus colonization of maize roots. *Plant and Soil*, **328**, 507–521.
York, L.M., Nord, E.A. & Lynch, J.P. (2013) Integration of root phenes for soil resource acquisition. *Frontiers in Plant Science*, **4**, 355.

Received 10 July 2016; accepted 7 October 2016
Handling Editor: Martin Nuñez

Supporting Information

Additional Supporting Information may be found in the online version of this article.

Appendix S1. Papers included in meta-analysis, map of study locations and sensitivity analysis.

Fig. S1. Map of locations of all studies included in the meta-analysis of cover crop and tillage effects on AMF colonization of cash crop roots.

Fig. S2. Boxplots of AMF colonization of cash crop roots from all field trials.