This item is the archived peer-reviewed author-version of:

Mycorrhizal fungi associated with high soil N:P ratios are more likely to be lost upon conversion from grasslands to arable agriculture

Reference:
Verbruggen Erik, Xiang Dan, Chen Baodong, Xu Tianle, Rillig Matthias C..- Mycorrhizal fungi associated with high soil N:P ratios are more likely to be lost upon conversion from grasslands to arable agriculture
Full text (Publishers DOI): http://dx.doi.org/doi:10.1016/j.soilbio.2015.03.008
Title: Mycorrhizal fungi associated with high soil N:P ratios are more likely to be lost upon conversion from grasslands to arable agriculture

Authors: Erik Verbruggen\textsuperscript{1,2}, Dan Xiang\textsuperscript{3,4}, Baodong Chen\textsuperscript{3,*}, Tianle Xu\textsuperscript{3}, Matthias C. Rillig\textsuperscript{1,2}

\textsuperscript{1}Dahlem Center of Plant Sciences, Plant Ecology, Freie Universität Berlin-Institut für Biologie, Berlin, Germany
\textsuperscript{2}Berlin-Brandenburg Institute of Advanced Biodiversity Research (BBIB), Berlin, Germany
\textsuperscript{3}State Key Laboratory of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, China
\textsuperscript{4}College of Resources and Environment, Qingdao Agricultural University, Qingdao, China

*Author for correspondence: Baodong Chen
Tel: +86 10 62849068; Fax: +86 10 62923549; Email: bdchen@rcees.ac.cn

Number of:
Text pages: 11, including 1 cover page and 2 pages with table and figure legends.
Figures: Totally 2 figures
Tables: Totally 1 table
References: 25
Abstract

Agriculture often leads to altered composition and reduced diversity of arbuscular mycorrhizal fungal (AMF) communities compared to semi-natural grassland systems. However, the ecology of taxa that are lost in this transition has thus far not been characterized to great depth. Here, we present an evaluation of the association of taxa that were reduced or lost in farmlands compared to grasslands with various environmental predictors within their native, semi-natural grasslands. These taxa were found to have a tendency to have higher positive correlations with soil N:P ratio and silt-content than taxa that were not significantly reduced in farmlands. In a permutation test it was found that for soil N:P ratio associations of those grassland-taxa were outside the 99% confidence interval of associations of random communities; this indicates that taxa that prevail at high N:P ratio are the ones most sensitive to agriculture. Because this environment is also commonly argued to impose the highest AMF benefit to plants, owing to a high plant P demand and consequent proliferation of high-quality AMF, the observation that those taxa are lost could indicate that agricultural fields are left with communities of reduced symbiotic quality.

Keywords: Arbuscular mycorrhiza; Indicator species; Maize; Grasslands; Mutualistic quality

1. Introduction

Arbuscular mycorrhizal fungi (AMF) are important and ubiquitous symbionts of the majority of land plants, and there exists great scientific interest in understanding their ecology and evolution (Johnson et al., 2006; Parniske, 2008). One of the most commonly found benefits AMF provide to plants is improved provision of soil phosphorus (Smith and Read, 2008) which, from a plant perspective, can often be more efficiently taken up by fine fungal hyphae than by relatively expensive plant roots (Fitter and Moyersoen, 1996). For the fungal partners the interaction is obligate as they solely rely on plant photosynthates for their carbon metabolism, but the extent to which they provide plants with P varies between AMF and according to environmental circumstances (Hammer et al., 2011; Kiers et al., 2011).

Given the high prevalence of this symbiosis across land plants, including many of the most abundant agricultural crops such as maize, rice, potato, and soybeans, there is concern whether common agricultural practices may reduce the benefit derived from AM symbiosis (e.g. Helgason et al., 1998).
Agriculture in general may impose strong selection pressures on AMF, such as tillage disrupting soil-borne mycelia, while also crop monoculture, frequent fallows, and application of fertilizers, pesticides and herbicides may adversely affect some AMF (Verbruggen and Kiers, 2010). Indeed, AMF communities are commonly found to change in response to agriculture in terms of community composition and diversity (Alguacil et al., 2008; Oehl et al., 2003). These changes can be accompanied by a change in AMF traits, such as faster spore-production and thus a reduced reliance on soil mycelium for survival over time (Oehl et al., 2009; Ohsowski et al., 2014), which may also correlate with the effects of these AMF on plant nutrient uptake (Hart and Reader, 2002). If such general shifts in traits occur, it would be expected that taxa typical of agricultural and natural systems would also exhibit different associations with abiotic and biotic environmental circumstances within these habitat types, because different traits would be favored under these different environments.

Until now no study has tested whether those taxa that differ between agricultural and natural ecosystems also differ in relationships with environmental circumstances in their “natural” habitats. This would be informative because it reveals whether there are commonalities among those taxa other than the tendency to be reduced under agriculture, and can even provide an indication which AMF attributes are favored in agricultural systems. Thus, the research question we ask here is whether the taxa that are reduced upon conversion from grasslands to farmland are associated with different environmental predictors than the other taxa. To answer this question we tested whether taxa that are significantly overrepresented in grasslands compared to farmlands (“grassland indicator species”) are more strongly correlated with certain environmental predictors than other taxa within grassland sites only.

2. Materials and Methods

The study comprised a large semi-arid ecoregion in northern China, where soil samples were taken from 50 agricultural fields cropping maize and 50 natural grasslands in the vicinity (< 2 km) of each of these agricultural fields. From each composite soil sample representing one field, DNA was isolated and AMF community composition was assessed by 454 pyrosequencing of PCR amplified 18S rRNA sequences in a nested approach representing the NS31-AM1 region commonly used in AMF research (e.g. Öpik et al., 2014). Various physico-chemical attributes of soils were determined and plant diversity was estimated in the grasslands in three random transects. These data have been previously published in Xiang et al. (2014), containing the full description of specific information on sampling and measurements.
For testing the relationship between field-specific variables and AMF communities, we chose those that are commonly implicated in structuring AMF community composition: soil available N (AN, mg kg$^{-1}$), soil available P (AP mg kg$^{-1}$), soil organic carbon (SOC, g kg$^{-1}$), percentage silt (silt %; this variable correlated strongly with percentage sand or clay), total N (%), plant diversity (Shannon H index), as well as soil N:P ratio, which was calculated by dividing AN by AP. The latter two variables and soil N:P ratio were log transformed to reduce skew based on visual assessment of distribution of values across sites prior to analysis (later referred to as log-). Most of these predictors were previously found to be significantly correlated with AMF community dissimilarity across grasslands and farmlands as presented in Xiang et al. (2014). For these environmental predictors it was tested whether the correlation of community dissimilarity (Bray-Curtis distance) with predictor-dissimilarity was higher for the set of 19 grassland “indicator-species” (as identified in (Xiang et al., 2014) according to indicator species analysis (De Cáceres and Legendre, 2009)) than for 19 randomly sampled OTUs using a bootstrapping procedure: random sampling was repeated 1000 times and the 99% and 95% confidence intervals were calculated against which the community-subset of indicators was compared. Additionally, single Pearson correlations between predictors and individual taxa were calculated to assess consistency of correlations across indicator taxa and other taxa. All statistics were performed using the “Vegan” (Oksanen et al., 2013) and “Indicspecies” (De Cáceres and Legendre, 2009) package version 1.7.2 in R version 3.0.3.

3. Results

Correlation coefficients of indicator taxa were consistently positive for N:P ratio and silt %, while for other predictors and for non-indicator taxa this was not the case (Fig. 1). The variables silt percentage and log N:P were correlated ($r=0.46; P < 0.001$), and thus potentially represent the same underlying association of taxa with the environment. However, the correlation coefficients of indicator taxa with these variables showed no correlations among each other ($r = 0.20; P = 0.41$). Thus, despite their correlation and similar effect, taxa tend to respond differently to these two predictors.

As a rigorous statistical test, the bootstrapping procedure revealed that the community-subset consisting of the indicator-taxa in grasslands was only more strongly correlated than random
community-subsets in the case of N:P ratio, while for all other predictors the correlation coefficient fell
within the 99% confidence interval of randomly selected taxa (Table 1). This was also true when
compared against the 95% confidence interval (not shown). When communities across both grasslands
and farmlands were combined, none of the indicator-taxa were significantly more strongly correlated
than random taxa (Table 1).

Within the grasslands, the number of indicator taxa found in soil was positively associated with N:P
ratio, while for the other taxa there was no significant association (Fig. 2a). This indicates that the
proportion of indicator taxa present in soil tended to increase with soil N:P ratio, and thus with
stoichiometric P limitation. In order to estimate the contribution of individual (indicator-) taxa to the
association between soil N:P ratio and AMF community dissimilarity, it was calculated how removing
each individual taxon from the community affects the correlation between these two variables (Fig. 2b).
As can be seen, for relatively many of the indicator taxa removal led to a reduction in the correlation,
while this was only true for a small proportion of the other taxa; for some of the other taxa removal
actually increased the correlation coefficient.

4. Discussion

The subset of AMF taxa that is reduced in agricultural systems compared to grasslands was found to be
strongly associated with soil N:P ratio in their “native” grassland habitat. There are two potential, non-
mutually exclusive, explanations for this finding. One is that the difference in soil N:P ratio between
grasslands and farmlands is a very strong driver of AMF community composition in those two systems.
Indeed, Xiang et al. (2014) found soil P levels to be a strong driver of AMF richness across the farmlands
and grasslands. Also in other large-scale studies comparing AMF across grasslands and farmlands, soil
physico-chemical variables have been found to be among the predominant drivers of AMF communities
(e.g. Jansa et al., 2014). However even though across farmlands and grasslands AMF community
composition was indeed also correlated with N:P ratio, this was not true to a greater extent for the
indicator taxa than for a randomly sampled community. This could be because these taxa are already at
relatively low abundance in the farmlands, and thus do not respond strongly to variation within the
farmland systems, which reduces the strength of the overall correlation.
An alternative or additional explanation is that AMF traits favored in farmlands are correlated with their performance under high soil N:P ratios. This can occur when traits that allow AMF to persist at low N:P ratios in grassland systems, predispose them towards being successful in farmlands with frequent tillage and fallows. Plausible candidate traits would include coping with low plant carbon supply such as reduced investment in nutrient-scavenging hyphae and higher allocation towards resting structures (e.g. Chagnon et al. 2013). Even though abundance, diversity, and community composition of AMF are often found to be affected by nitrogen and/or phosphorus applications (e.g. Chen et al., 2014), research on functional properties of AMF favored under different soil nutrient levels are relatively scarce. In one study, AMF from long-term agricultural plots that were artificially deficient in nutrients (N and P) have been found to provide reduced growth benefit to plants compared to AMF from control plots (Antunes et al., 2012). In another study AMF applied as soil inoculum from fertilized systems where nutrients were far from limiting have also been found to exhibit reduced beneficial effects to plants compared to un-fertilized controls (Johnson, 1993). Such effects are consistent with the idea that under both extremely low and high nutrient levels the AMF community is enriched with low-quality mutualists. This happens because under these conditions no AMF can provide a nutritional benefit, which relaxes the incentive for and/or possibility of plants to distinguish and favor high-quality mutualists, providing a relative advantage to low-quality mutualists (Steidinger and Bever, 2014). Furthermore, the effects of nutrient limitation on AMF-plant interactions have been shown to be to a large extent driven by the N:P ratio of nutrients at the plant’s disposal (Johnson, 2010), indicating that in particular relative P limitation determines the extent to which plants rely on AMF for nutrition. Together, these ideas and observations would suggest that AMF in soils with increasing soil N:P ratio are under stronger pressures to provide P to hosts. In this light our observation that taxa that are found particularly under those conditions are reduced under agriculture would indicate that agricultural systems harbor a community consisting of less beneficial AMF with regard to P provision.

As can be seen in Table 1 the random selection of 19 taxa generally confirmed the Mantel tests of significance of all taxa in that when the 99% CI did not overlap with zero, Mantel tests also showed a significant correlation at the $P < 0.01$ level. This indicates that this procedure was able to estimate the CI to a similar extent as a Mantel test and thus allowing us to compare the 19 indicator taxa against an appropriate null model. This was true for all but one exception: soil silt % in the grasslands only. Here, the Mantel test of the full community was not significant, while selection of 19 random taxa led to correlations higher than zero in more than 99% of cases (Table 1). This is probably caused by the fact
that there is a relatively high consistency across fungal taxa in responding to silt %, hence values larger than zero in many subsets, but generally small effects, leading to a small correlation overall and thus not being significant at the $P < 0.05$ level.

Further research effort is now needed to test whether the affinity of some taxa to a relatively high soil N:P ratio we observed in this study system is a common phenomenon, and whether this indeed correlates with functional attributes of these taxa. Unfortunately, it is hard to compare measurements of available soil nutrient contents across studies because of e.g. differences in protocols. In case of dividing two of them, as is the case for soil N:P ratio, this problem may even be exacerbated. The same is true for comparing OTU occurrence across studies, where differences in lab analysis and bioinformatics may reduce comparability across datasets. For this reason the current study is restricted to a single dataset, which does however represent a relatively large and ecologically relevant area of grasslands and agricultural fields in Northern China (Xiang et al., 2014). It would be of great interest to confirm whether the relationship between AMF and soil N:P, and their sensitivity to agriculture, is also found in other climatic regions, ecosystem types, as well as crops other than maize as in the current study. Understanding which functional traits in AM fungal communities, if any, are lost in agricultural systems may help inform improved management practices, such as fertilization regimes. Clearly, the need for “sustainable intensification” (Loos et al., 2014) in agriculture to feed a growing human population highlights the urgency to address such questions.

Acknowledgements

This research was financially supported by National Natural Science Foundation of China (41071178; 41371264), Chinese Academy of Sciences (XDB15030100), and a Joint Project of the Ministry of Environmental Protection, P.R. China and the Chinese Academy of Sciences (STSN-21-04). EV and MCR were supported by the Deutsche Forschungsgemeinschaft.

References


Table 1 Pearson r and statistical significance of Mantel tests of either all taxa, indicator taxa (“grassland indicator taxa”) only, in either the entire data set of farmlands and grasslands or grasslands only. A bold value indicates that the correlation of indicator taxa is higher than for randomly selected subsamples (“99% confidence interval random selection”).

<table>
<thead>
<tr>
<th>environmental variable</th>
<th>all taxa</th>
<th>99% confidence interval</th>
<th>grassland indicator taxa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pearson r</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>0.199</td>
<td>0.008</td>
<td>-0.016 to 0.280</td>
</tr>
<tr>
<td>SOC</td>
<td>0.089</td>
<td>&gt;0.05</td>
<td>-0.057 to 0.229</td>
</tr>
<tr>
<td>plant diversity</td>
<td>ND(^1)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Log N</td>
<td>0.153</td>
<td>0.001</td>
<td>-0.009 to 0.181</td>
</tr>
<tr>
<td>Log P</td>
<td>0.125</td>
<td>0.001</td>
<td>0.023 to 0.175</td>
</tr>
<tr>
<td>total N</td>
<td>0.052</td>
<td>&gt;0.05</td>
<td>-0.073 to 0.229</td>
</tr>
<tr>
<td>Log N:P</td>
<td>0.104</td>
<td>0.001</td>
<td>-0.005 to 0.194</td>
</tr>
<tr>
<td>silt %</td>
<td>0.141</td>
<td>0.002</td>
<td>0.012 to 0.188</td>
</tr>
</tbody>
</table>

**grasslands only**

<table>
<thead>
<tr>
<th>environmental variable</th>
<th>all taxa</th>
<th>99% confidence interval</th>
<th>grassland indicator taxa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pearson r</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>0.182</td>
<td>0.025</td>
<td>-0.007 to 0.257</td>
</tr>
<tr>
<td>SOC</td>
<td>0.038</td>
<td>&gt;0.05</td>
<td>-0.067 to 0.190</td>
</tr>
<tr>
<td>plant diversity</td>
<td>0.050</td>
<td>&gt;0.05</td>
<td>-0.029 to 0.141</td>
</tr>
<tr>
<td>Log N</td>
<td>0.225</td>
<td>0.004</td>
<td>0.061 to 0.250</td>
</tr>
<tr>
<td>Log P</td>
<td>0.298</td>
<td>0.004</td>
<td>0.060 to 0.350</td>
</tr>
<tr>
<td>total N</td>
<td>0.017</td>
<td>&gt;0.05</td>
<td>-0.072 to 0.175</td>
</tr>
<tr>
<td>Log N:P</td>
<td>0.279</td>
<td>0.005</td>
<td>0.061 to 0.348</td>
</tr>
<tr>
<td>silt %</td>
<td>0.113</td>
<td>&gt;0.05</td>
<td>0.062 to 0.357</td>
</tr>
</tbody>
</table>

\(^1\)ND – not determined.
Figure legends

**Fig. 1.** Pearson correlation coefficients of 19 grassland indicator-taxa (red circles) and all other 71 taxa (blue diamonds) with different environmental predictors across the fifty grassland sites. In the bottom right the average relative abundance of the same groups is plotted across sites, as an indication of their abundance compared to all other taxa.

**Fig. 2.** Relationship between log N:P ratio and number of taxa at each grassland site (a), distinguished in indicator taxa (red circles, solid line) and all other taxa (blue diamonds, dashed line). Linear regression reveals that log N:P significantly predicts the indicator taxa ($y = 7.78x - 0.89; r^2 = 0.32; P < 0.001$) but that this is not true for the other taxa ($y = 3.40x + 13.75; r^2 = 0.01; P = 0.20$). The lower panel (b) indicates the contribution of individual taxa to the relationship between community dissimilarity and soil N:P ratio. On the x-axis the point-biserial correlation coefficient (correlation where one variable is dichotomous) of taxa in indicator species analysis is shown, which is a measure of their “indicator strength” (De Cáceres and Legendre, 2009). On the y-axis, the Pearson correlation coefficient of the relationship between communities (based on presence-absence based Jaccard index) and dissimilarity in N:P ratios is shown while each time dropping another taxon. The dashed line is added to indicate the correlation coefficient using the entire community, and thus when values on the y-axis are lower than this line the dropped taxa positively contribute to the correlation between soil N:P ratio and community compositions.
Fig. 1.
Fig. 2.