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

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

6

7 Authors: **Erik Verbruggen<sup>1,2</sup>, Dan Xiang<sup>3,4</sup>, Baodong Chen<sup>3,\*</sup>, Tianle Xu<sup>3</sup>, Matthias C. Rillig<sup>1,2</sup>**

8 <sup>1</sup>Dahlem Center of Plant Sciences, Plant Ecology, Freie Universität Berlin-Institut für Biologie, Berlin,  
9 Germany

10 <sup>2</sup>Berlin-Brandenburg Institute of Advanced Biodiversity Research (BBIB), Berlin, Germany

11 <sup>3</sup>State Key Laboratory of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences,  
12 Chinese Academy of Sciences, Beijing, China

13 <sup>4</sup>College of Resources and Environment, Qingdao Agricultural University, Qingdao, China

14

15 \*Author for correspondence: Baodong Chen

16 Tel: +86 10 62849068; Fax: +86 10 62923549; Email: bdchen@rcees.ac.cn

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25

26 **Abstract**

27

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

41

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

43

44 **1. Introduction**

45

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

54

55 Given the high prevalence of this symbiosis across land plants, including many of the most abundant  
56 agricultural crops such as maize, rice, potato, and soybeans, there is concern whether common  
57 agricultural practices may reduce the benefit derived from AM symbiosis (e.g. Helgason et al., 1998).

58 Agriculture in general may impose strong selection pressures on AMF, such as tillage disrupting soil-  
59 borne mycelia, while also crop monoculture, frequent fallows, and application of fertilizers, pesticides  
60 and herbicides may adversely affect some AMF (Verbruggen and Kiers, 2010). Indeed, AMF communities  
61 are commonly found to change in response to agriculture in terms of community composition and  
62 diversity (Alguacil et al., 2008; Oehl et al., 2003). These changes can be accompanied by a change in AMF  
63 traits, such as faster spore-production and thus a reduced reliance on soil mycelium for survival over  
64 time (Oehl et al., 2009; Ohsowski et al., 2014), which may also correlate with the effects of these AMF  
65 on plant nutrient uptake (Hart and Reader, 2002). If such general shifts in traits occur, it would be  
66 expected that taxa typical of agricultural and natural systems would also exhibit different associations  
67 with abiotic and biotic environmental circumstances within these habitat types, because different traits  
68 would be favored under these different environments.

69

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

79

## 80 **2. Materials and Methods**

81

82 The study comprised a large semi-arid ecoregion in northern China, where soil samples were taken from  
83 50 agricultural fields cropping maize and 50 natural grasslands in the vicinity (< 2 km) of each of these  
84 agricultural fields. From each composite soil sample representing one field, DNA was isolated and AMF  
85 community composition was assessed by 454 pyrosequencing of PCR amplified 18S rRNA sequences in a  
86 nested approach representing the NS31-AM1 region commonly used in AMF research (e.g. Öpik et al.,  
87 2014). Various physico-chemical attributes of soils were determined and plant diversity was estimated in  
88 the grasslands in three random transects. These data have been previously published in Xiang et al.  
89 (2014), containing the full description of specific information on sampling and measurements.

90

91 For testing the relationship between field-specific variables and AMF communities, we chose those that  
92 are commonly implicated in structuring AMF community composition: soil available N (AN, mg kg<sup>-1</sup>), soil  
93 available P (AP mg kg<sup>-1</sup>), soil organic carbon (SOC, g kg<sup>-1</sup>), percentage silt (silt %; this variable correlated  
94 strongly with percentage sand or clay), total N (%), plant diversity (Shannon H index), as well as soil N:P  
95 ratio, which was calculated by dividing AN by AP. The latter two variables and soil N:P ratio were <sup>10</sup>log  
96 transformed to reduce skew based on visual assessment of distribution of values across sites prior to  
97 analysis (later referred to as log-). Most of these predictors were previously found to be significantly  
98 correlated with AMF community dissimilarity across grasslands and farmlands as presented in Xiang et  
99 al. (2014).

100

101 For these environmental predictors it was tested whether the correlation of community dissimilarity  
102 (Bray-Curtis distance) with predictor-dissimilarity was higher for the set of 19 grassland “indicator-  
103 species” (as identified in (Xiang et al., 2014) according to indicator species analysis (De Cáceres and  
104 Legendre, 2009)) than for 19 randomly sampled OTUs using a bootstrapping procedure: random  
105 sampling was repeated 1000 times and the 99% and 95% confidence intervals were calculated against  
106 which the community-subset of indicators was compared. Additionally, single Pearson correlations  
107 between predictors and individual taxa were calculated to assess consistency of correlations across  
108 indicator taxa and other taxa. All statistics were performed using the “Vegan” (Oksanen et al., 2013) and  
109 “Indicspecies” (De Cáceres and Legendre, 2009) package version 1.7.2 in R version 3.0.3.

110

### 111 **3. Results**

112

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

119

120 As a rigorous statistical test, the bootstrapping procedure revealed that the community-subset  
121 consisting of the indicator-taxa in grasslands was only more strongly correlated than random

122 community-subsets in the case of N:P ratio, while for all other predictors the correlation coefficient fell  
123 within the 99% confidence interval of randomly selected taxa (Table 1). This was also true when  
124 compared against the 95% confidence interval (not shown). When communities across both grasslands  
125 and farmlands were combined, none of the indicator-taxa were significantly more strongly correlated  
126 than random taxa (Table 1).

127

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

137

#### 138 **4. Discussion**

139

140 The subset of AMF taxa that is reduced in agricultural systems compared to grasslands was found to be  
141 strongly associated with soil N:P ratio in their “native” grassland habitat. There are two potential, non-  
142 mutually exclusive, explanations for this finding. One is that the difference in soil N:P ratio between  
143 grasslands and farmlands is a very strong driver of AMF community composition in those two systems.  
144 Indeed, Xiang *et al.* (2014) found soil P levels to be a strong driver of AMF richness across the farmlands  
145 and grasslands. Also in other large-scale studies comparing AMF across grasslands and farmlands, soil  
146 physico-chemical variables have been found to be among the predominant drivers of AMF communities  
147 (e.g. Jansa *et al.*, 2014). However even though across farmlands and grasslands AMF community  
148 composition was indeed also correlated with N:P ratio, this was not true to a greater extent for the  
149 indicator taxa than for a randomly sampled community. This could be because these taxa are already at  
150 relatively low abundance in the farmlands, and thus do not respond strongly to variation within the  
151 farmland systems, which reduces the strength of the overall correlation.

152

153 An alternative or additional explanation is that AMF traits favored in farmlands are correlated with their  
154 performance under high soil N:P ratios. This can occur when traits that allow AMF to persist at low N:P  
155 ratios in grassland systems, predispose them towards being successful in farmlands with frequent tillage  
156 and fallows. Plausible candidate traits would include coping with low plant carbon supply such as  
157 reduced investment in nutrient-scavenging hyphae and higher allocation towards resting structures (e.g.  
158 Chagnon et al. 2013). Even though abundance, diversity, and community composition of AMF are often  
159 found to be affected by nitrogen and/or phosphorus applications (e.g. Chen et al., 2014), research on  
160 functional properties of AMF favored under different soil nutrient levels are relatively scarce. In one  
161 study, AMF from long-term agricultural plots that were artificially deficient in nutrients (N and P) have  
162 been found to provide reduced growth benefit to plants compared to AMF from control plots (Antunes  
163 et al., 2012). In another study AMF applied as soil inoculum from fertilized systems where nutrients  
164 were far from limiting have also been found to exhibit reduced beneficial effects to plants compared to  
165 un-fertilized controls (Johnson, 1993). Such effects are consistent with the idea that under both  
166 extremely low and high nutrient levels the AMF community is enriched with low-quality mutualists. This  
167 happens because under these conditions no AMF can provide a nutritional benefit, which relaxes the  
168 incentive for and/or possibility of plants to distinguish and favor high-quality mutualists, providing a  
169 relative advantage to low-quality mutualists (Steidinger and Bever, 2014). Furthermore, the effects of  
170 nutrient limitation on AMF-plant interactions have been shown to be to a large extent driven by the N:P  
171 ratio of nutrients at the plant's disposal (Johnson, 2010), indicating that in particular relative P limitation  
172 determines the extent to which plants rely on AMF for nutrition. Together, these ideas and observations  
173 would suggest that AMF in soils with increasing soil N:P ratio are under stronger pressures to provide P  
174 to hosts. In this light our observation that taxa that are found particularly under those conditions are  
175 reduced under agriculture would indicate that agricultural systems harbor a community consisting of  
176 less beneficial AMF with regard to P provision.

177  
178 As can be seen in Table 1 the random selection of 19 taxa generally confirmed the Mantel tests of  
179 significance of all taxa in that when the 99% CI did not overlap with zero, Mantel tests also showed a  
180 significant correlation at the  $P < 0.01$  level. This indicates that this procedure was able to estimate the CI  
181 to a similar extent as a Mantel test and thus allowing us to compare the 19 indicator taxa against an  
182 appropriate null model. This was true for all but one exception: soil silt % in the grasslands only. Here,  
183 the Mantel test of the full community was not significant, while selection of 19 random taxa led to  
184 correlations higher than zero in more than 99% of cases (Table 1). This is probably caused by the fact

185 that there is a relatively high consistency across fungal taxa in responding to silt %, hence values larger  
186 than zero in many subsets, but generally small effects, leading to a small correlation overall and thus not  
187 being significant at the  $P < 0.05$  level.

188  
189 Further research effort is now needed to test whether the affinity of some taxa to a relatively high soil  
190 N:P ratio we observed in this study system is a common phenomenon, and whether this indeed  
191 correlates with functional attributes of these taxa. Unfortunately, it is hard to compare measurements  
192 of available soil nutrient contents across studies because of e.g. differences in protocols. In case of  
193 dividing two of them, as is the case for soil N:P ratio, this problem may even be exacerbated. The same  
194 is true for comparing OTU occurrence across studies, where differences in lab analysis and  
195 bioinformatics may reduce comparability across datasets. For this reason the current study is restricted  
196 to a single dataset, which does however represent a relatively large and ecologically relevant area of  
197 grasslands and agricultural fields in Northern China (Xiang et al., 2014). It would be of great interest to  
198 confirm whether the relationship between AMF and soil N:P, and their sensitivity to agriculture, is also  
199 found in other climatic regions, ecosystem types, as well as crops other than maize as in the current  
200 study. Understanding which functional traits in AM fungal communities, if any, are lost in agricultural  
201 systems may help inform improved management practices, such as fertilization regimes. Clearly, the  
202 need for “sustainable intensification” (Loos et al., 2014) in agriculture to feed a growing human  
203 population highlights the urgency to address such questions.

204

## 205 **Acknowledgements**

206

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211

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- 274
- 275

276 **Table 1** Pearson r and statistical significance of Mantel tests of either all taxa, indicator taxa (“grassland  
 277 indicator taxa”) only, in either the entire data set of farmlands and grasslands or grasslands only. A bold  
 278 value indicates that the correlation of indicator taxa is higher than for randomly selected subsamples  
 279 (“99% confidence interval random selection”).  
 280

**farmlands and  
 grasslands**

<i>environmental variable</i>	<i>all taxa</i>		<i>99% confidence interval</i>		<i>grassland indicator taxa</i>
	Pearson r	<i>P</i>	<i>random selection</i>		
pH	0.199	0.008	-0.016	to 0.280	0.223
SOC	0.089	>0.05	-0.057	to 0.229	0.063
plant diversity	ND <sup>1</sup>		ND		ND
Log N	0.153	0.001	-0.009	to 0.181	0.121
Log P	0.125	0.001	0.023	to 0.175	0.041
total N	0.052	>0.05	-0.073	to 0.229	0.063
Log N:P	0.104	0.001	-0.005	to 0.194	0.110
silt %	0.141	0.002	0.012	to 0.188	0.099

**grasslands only**

<i>environmental variable</i>	<i>all taxa</i>		<i>99% confidence interval</i>		<i>grassland indicator taxa</i>
	Pearson r	<i>P</i>	<i>random selection</i>		
pH	0.182	0.025	-0.007	to 0.257	0.213
SOC	0.038	>0.05	-0.067	to 0.190	0.067
plant diversity	0.050	>0.05	-0.029	to 0.141	0.053
Log N	0.225	0.004	0.061	to 0.250	0.232
Log P	0.298	0.004	0.060	to 0.350	0.266
total N	0.017	>0.05	-0.072	to 0.175	0.071
Log N:P	0.279	0.005	0.061	to 0.348	<b>**0.362</b>
silt %	0.113	>0.05	0.062	to 0.357	0.294

281

282 <sup>1</sup>ND – not determined.

283

284 **Figure legends**

285

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

290

291 **Fig. 2.** Relationship between log N:P ratio and number of taxa at each grassland site (a), distinguished in  
292 indicator taxa (red circles, solid line) and all other taxa (blue diamonds, dashed line). Linear regression  
293 reveals that log N:P significantly predicts the indicator taxa ( $y = 7.78x - 0.89$ ;  $r^2 = 0.32$ ;  $P < 0.001$ ) but that  
294 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  
295 contribution of individual taxa to the relationship between community dissimilarity and soil N:P ratio.  
296 On the x-axis the point-biserial correlation coefficient (correlation where one variable is dichotomous) of  
297 taxa in indicator species analysis is shown, which is a measure of their “indicator strength” (De Cáceres  
298 and Legendre, 2009). On the y-axis, the Pearson correlation coefficient of the relationship between  
299 communities (based on presence-absence based Jaccard index) and dissimilarity in N:P ratios is shown  
300 while each time dropping another taxon. The dashed line is added to indicate the correlation coefficient  
301 using the entire community, and thus when values on the y-axis are lower than this line the dropped  
302 taxa positively contribute to the correlation between soil N:P ratio and community compositions.

Fig. 1.

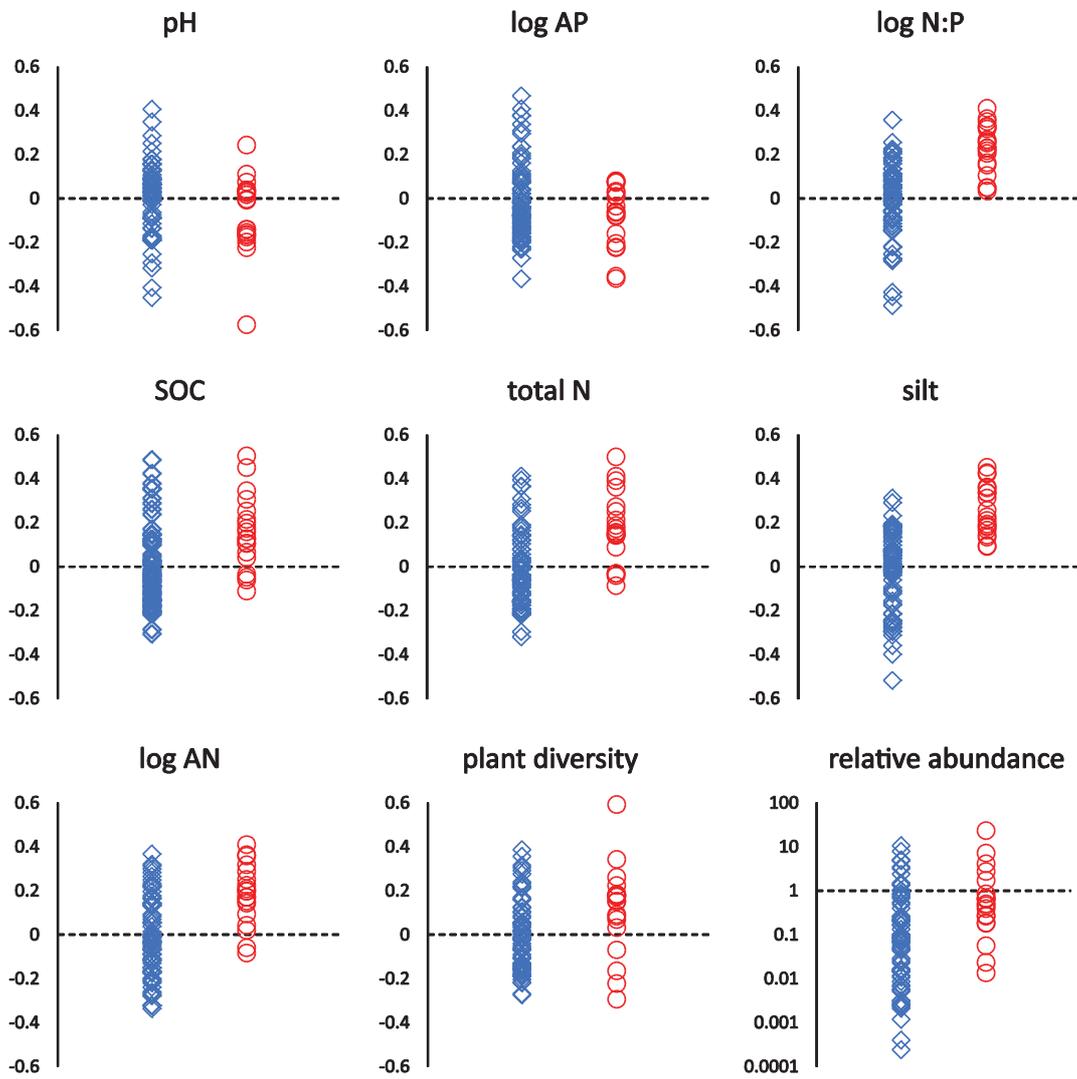


Fig. 2.

