

## Diversity of arbuscular mycorrhizal fungi in no-till and conventionally tilled vineyards

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### Summary

The use of a permanent vegetation cover or frequent tillage in vineyards may affect soil water budget, nutrient availability, soil compaction, soil erosion and soil microbe biodiversity, and through all these and other factors also yield and wine quality parameters. The abundance and diversity of arbuscular mycorrhizal fungi (AMF) might also be influenced, but so far effects on AMF by permanent vegetation cover (= no-tillage systems) or repeated chiseling and rotary-tilling have rarely been compared in vineyards. The objective of this on-farm study was to determine AMF species richness and diversity in two adjacent vineyards in Palatinate (SW Germany). In both vineyards, grown on fertile Luvisols, the var. "Pinot Gris" was grown for 39 years, but with different soil cultivation and different fertilization strategies. In one vineyard, soil was maintained periodically without vegetation by passing rotary cultivator and chiseling between the grapevine rows ('inter-rows') several times per year, preferably during spring and summer and in dependency of rainfall and 'weed' growth, and fertilization was mainly by organic fertilizers in the last ten years before soil sampling. In the other vineyard, a permanent vegetation has been established since planting, dominated by *Lolium perenne*, and mineral fertilizers were exclusively applied. Despite of similar high nutrient availability in both soils, in particular of phosphorus, astonishing high AMF species richness and diversity were found in both vineyards. In the no-tillage inter-rows, 34 AMF species were found, with a species composition typically for Central European permanent grasslands (Shannon diversity index 2.45). In the tillage system 24 AMF species were found with a composition as known for extensively used, cultivated Central European croplands (diversity index 2.26). We conclude that above all soil cultivation has affected AMF diversity in these Central European vineyards, while the level and type of fertilization affected the AMF communities only on a minor level.

**Key words:** grapes; green cover strategies; Glomeromycotina; mycorrhiza; soil management

### Introduction

Permanent vegetation cover or no-tillage between rows in vineyards has always been practiced in different grapevine regions. Permanent cover is common above all in sloped areas or in regions with relatively high annual precipitation, in order to prevent soil erosion and soil loss. In drier, semi-arid to arid, often Mediterranean areas, however, soils are periodically tilled or left completely bare during the vegetation period of the grapevine, similar to those in olive plantations (TURRINI et al., 2017). In such climates, natural grasses and herbs, as well as green cover plants have often been considered as grapevine 'weeds' competing for water and soil nutrients, and thus possibly affecting negatively grapevine yields and wine quality (RODRIGUEZ-LOVELLE et al., 2000; GUERRA and STEENWERTH, 2011). The management of such natural to intentionally seeded permanent vegetations

has become major importance, especially in respect to on-going or expected climatic changes (RUPP, 1996; KOCH and OEHL, 2018).

Arbuscular mycorrhizal fungi (AMF) of the fungal subphylum Glomeromycotina represent an important group of beneficial soil microorganisms that have positive effects on grapevine growth and health (PETGEN et al., 1997; SCHREINER, 2007; SCANDELLARI, 2017) as well as on soil aggregate formation and prevention of soil erosion (RILLIG and MUMEY, 2006). It can be assumed that they can also affect grapevine quality positively, especially in drier years or in more arid regions, where water periodically is scarce and might restrict optimum grapevine development. However, these fungi are obligatory biotroph, which means that they need living plants for their maintenance at their habitats (SMITH and READ, 2008). Keeping the vineyard soil free of weeds and grassland plant species, as it is sometimes practiced not only in the grapevine rows, but also in the whole inter-spaces ('inter-rows'), must thus affect development and reproduction of this fungal group, although in such situations they may remain important symbiotic partners of the deep-rooting grapevine plants.

The objective of the present study was to determine the species richness and diversity of AM fungi in two adjacent vineyards, but applying different soil cultivation and fertilization strategies. The vineyards, both grown for 39 years with the white wine variety "Pinot Gris", were located in Palatinate (Southwestern Germany), which represents one of the most traditional, largest wine growing areas in the North of the Alps, where grapevine has been produced since the Roman times (SCHULTZ and JONES, 2010). It represents also one of the driest and warmest regions in Germany (KOCH and OEHL, 2018), climatically belonging to the European temperate zone, but both with an Atlantic, and also a continental influence, well documented by the presence of *Castanea sativa* L. grown on Cambisols and of Tschernosem/Phaeosem soils in close vicinity to each other. Our results may help to better understand and improve viticultural systems, increase natural soil fertility and biologically mediated nutrient availability, and to increase soil microbes biodiversity in vineyards.

### Material and methods

#### Study site and soil sampling

The two vineyards are located at about 700 m southeast of the village Hainfeld (Palatinate, Germany; 49°15'16"N; 8°06'24"E) established on Haplic Luvisol soils according FAO/IUSS soil classification. Mean annual temperature is approximately 11.5°C, and mean annual rainfall is ca. 670 mm in a bi-modal distribution, with generally less rainfall in spring and early autumn than during summer and winter (KOCH and OEHL, 2018). The rootstocks with grafted "Pinot Gris" were planted in rows in June 1975, choosing 2.0 m distances between the rows and 1.20 m within the rows. Differences between both vineyards were in soil cultivation strategy of the inter-rows of the grapevine plants, which was no-tillage in one vineyard since the first year of planting, while in the other vineyard, the soil was periodically cultivated by rotary cultivator or chisel several times per year, depending on rainfall and 'vineyard weed' growth. The use of synthetic pesticides for plant protection against pathogens and pests

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was similar in both vineyards during the 39 years and was carried out according to good agricultural practice. No herbicides were used beneath the rootstocks in the last 10 years before soil sampling, while before herbicides were mainly based on glyphosate or glufosinate, and before 1985 also on paraquat and diquat. Both vineyards received about the same quantity of fertilizers according to the regional recommendations for grapevine production (since the year 2000 approx. 50–60 kg N, 10 kg P, 60–80 kg K, 15 kg Mg ha<sup>-1</sup> a<sup>-1</sup>). However, the tilled vineyard received composted horse manure, derived from an agricultural farm situated about 5 km west to the vineyards, instead of mineral fertilizers, which were applied in the no-tillage vineyard. Soil sampling (0–10 cm depth) was performed in early springtime 2014 in five selected plots of 5×20 m<sup>2</sup> per vineyard as described in OEHL et al. (2005b) in the middle of the inter-rows at about 1.0 m distance to the Pinot Gris rootstocks; this represented five replicates per site. In each replicate plot, six subsamples were taken and pooled, totaling in about 1.2 kg soil per plot. Soil samples were carefully air-dried, sieved at 5 mm and stored as described in OEHL et al. (2005b), before they were analyzed for selected chemical soil parameters and AMF spore density and diversity.

### AMF spore isolation and identification

AMF spores were extracted from the soil samples by wet sieving and sucrose density gradient centrifugation (SIEVERDING, 1991). Spores, spore clusters and sporocarps were picked from the extractions in Petri dishes without pre-selection using a Leica S8APO and mounted together on microscope slides using polyvinyl-lactic acid-glycerol (PLVG) or PLVG mixed 1:1 (v/v) with Melzer's reagent. The slides were systematically examined under a Leica DM750 compound microscope at up to 400-fold magnification to identify and count all morphologically distinct AMF spore types present. Morphological spore identification was based on two identification manuals (SCHENCK and PÉREZ, 1990; BŁASZKOWSKI, 2012) and all newer existing AMF species descriptions. Taxonomic classification was based on the Glomeromycota system of OEHL et al. (2011) published by the International Mycological Association (IMA), with a few updates (e.g. SIEVERDING et al., 2014; BŁASZKOWSKI et al., 2015; 2017; Oehl et al., 2016).

### Statistical analyses

Student t-test was applied to test for significant differences between the AMF populations in the two vineyards in AMF spore density, species richness and diversity, and for spore abundance for each AMF species found in both vineyards.

## Results and discussion

### Chemical soil parameters

Both vineyards had quite similar chemical soil parameters (Tab. 1) with an adequate organic carbon content (24.6–26.1 g kg<sup>-1</sup>) and slightly acidic to neutral pH values (6.6–6.8, measured in 1n KCl, 7.0–7.4 in water), which corresponds well with the similar level of nutrient application, despite of using quite different types of fertilizers. Available nutrient values were all classified as high to very high and reflect the excessive fertilization recommendations of the past, especially before 1985. In particular, phosphorus levels were extremely high so that low AMF spore density, species richness and diversity could be expected, since it is generally assumed that at higher plant available P levels plants depend less on mycorrhiza (SMITH and READ, 2008). The values also suggest that P fertilization would not be needed, or should be further reduced in the next years, and the same might apply also for K and Mg fertilization. The relatively high copper contents in both soils may reflect the intensive use of copper-based fungicides

**Tab. 1:** Chemical soil parameters in no-tillage and periodically tilled inter-rows of two 39-years old “Pinot Gris” vineyards

Soil parameter	No-till	Tillage	Unit	Nutrient availability level according to current recommendations in Germany
Organic Carbon	26.1	24.6	g kg <sup>-1</sup>	
pH (H <sub>2</sub> O)	7.0	7.4		
pH (1n KCl)	6.6	6.8		
Humus (%)	45	42	g kg <sup>-1</sup>	
Ca	4.6	3.5	g kg <sup>-1</sup>	Medium
P (Na-Acetat)*	69	104	mg kg <sup>-1</sup>	Very high
P (DL)*	203	257	mg kg <sup>-1</sup>	Very high
P (Citrat)*	428	467	mg kg <sup>-1</sup>	Very high
K (Na-Acetat)	415	479	mg kg <sup>-1</sup>	Very high
K (DL)	515	562	mg kg <sup>-1</sup>	Very high
Mg (DL)	315	246	mg kg <sup>-1</sup>	Very high
Cu	43	43	mg kg <sup>-1</sup>	Very high
Zn	112	35	mg kg <sup>-1</sup>	High

\*Based on different methods to determine P-availability in European soils (NEYROUD et al., 2003).

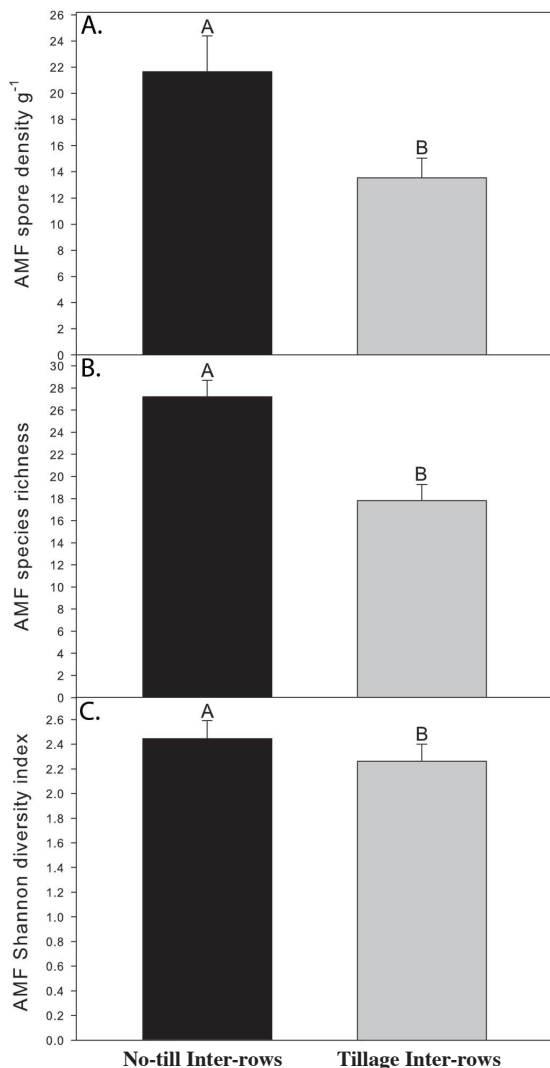
during the last hundred years of grapevine production in order to suppress downy mildew, which – together with powdery mildew – has been the major pathogen in the grapevine growing areas of the region during the last century.

### AMF spore density

In the no-tillage inter-rows, AMF spore density was approximately 60% higher (22 g<sup>-1</sup> soil) than in the periodically cultivated inter-rows (14 g<sup>-1</sup> soil, Fig. 1A). These values represent similar numbers for the no-till inter-rows, when compared to values from permanent grasslands studied in the Upper Rhine region between Basel and Mainz, where 15–40 spores g<sup>-1</sup> soil were found in de-carbonated soils in early spring (e.g. OEHL et al., 2003, 2005b, 2010, 2017). The spore densities found in the cultivated inter-rows were slightly above the densities reported for conventionally or organically managed croplands of similar soil pH in Central Europe (OEHL et al., 2003; MAURER et al., 2014). One reason for this finding might be that soil treatment by vineyard chisels and rotary cultivators is generally shallower (5–15 cm) than by ploughing in arable lands (generally 15–25 cm in low input croplands, in the past sometimes up to 25–30 cm in croplands; OEHL et al., 2005b). Thus, the sub-soil AMF hyphal network and AMF communities in vineyards might have been less disturbed than in croplands (OEHL et al., 2005b). The high fertilization levels did not lead to low spore densities in the two vineyards. Soil cultivation had a stronger effect than nutrient availability on AMF spore density, which is in accordance with AVIO et al. (2013) and SALE et al. (2015), and in general vineyards might have higher spore densities than croplands, which is in accordance with OEHL et al. (2005b).

### AMF species richness and diversity

Total AMF species richness was about 40% higher in the no-tillage (34 species) than in the cultivated inter-rows (24 species, Tab. 2), and mean species richness per plot was approximately 50% higher in the no-tillage vineyard (27 versus 18 species; Fig. 1B). Moreover, AMF



**Fig. 1:** AMF spore density, species richness and Shannon-Weaver diversity in no-tillage and conventional tillage inter-rows of two 39-years old “Pinot Gris” vineyards.

diversity expressed as the Shannon index (H-value) was also higher in the no-tillage (2.45) than in the cultivated inter-rows (2.26; Fig. 1 C). This is in accordance with LUMINI et al. (2010), who found higher AMF diversity in pastures and covered vineyards than in tilled vineyards in a Mediterranean environment of Italy. Our species numbers and diversity indices are remarkable, as they are, despite of the high nutrient availability values, in the same range of numbers found in extensively managed grasslands (26-39 species and 2.29-2.54 H-value) and extensively to intensively managed, cultivated croplands (21-28 and 1.72-2.45) in Central Europe (OEHL et al., 2003, 2010; MAURER et al., 2014; WETZEL et al., 2014; SÄLE et al., 2015). Those sites had much lower nutrient availability values. So far, the opinion was that high nutrient availability would counteract the development and diversity of AMF. Our new data suggest that many, if not all AMF species, naturally occurring in extensively managed or natural permanent grasslands can somehow litigate with high nutrient (and even copper) values also in permanent covered inter-rows of vineyards. They will not disappear under grapevine production, especially not in inter-rows with permanent green cover. This is encouraging, as there is no measurable loss of AMF biodiversity in vineyards, as a clear difference to intensively used croplands (e.g. OEHL et al., 2003, 2010). We found, however, low spore densities and species richness of Acaulosporaceae, Ambisporaceae and Gigasporales species (in to-

**Tab. 2:** Spore density for AMF species in no-till and periodically tilled inter-rows of two “Pinot Gris” vineyards

AMF species	Spore density per 100 g (standard deviation)		p-value
	No tilled	Tilled	
<b>Equal abundant in both vineyards</b>			
<i>Claroideoglossum luteum</i>	18 (1.4)	18 (3.0)	0.500
<i>Rhizoglossum fasciculatum</i>	10 (0.7)	10 (1.6)	0.500
<i>Glomus spinosum</i>	2 (0.2)	2 (0.3)	0.500
<i>Rhizoglossum invermaium</i>	30 (2.2)	22 (3.8)	0.333
<i>Funneliformis mosseae</i>	46 (3.5)	35 (6.0)	0.263
<i>Claroideoglossum claroideum</i>	15 (1.2)	10 (1.8)	0.118
<b>More abundant in the periodically tilled vineyard</b>			
<i>Glomus diaphanum</i>	21 (1.6)	112 (19.2)	<b>0.005</b>
<i>Archaeospora trappei</i>	38 (2.9)	58 (10.0)	0.092
<b>More abundant in the no-till vineyard</b>			
<i>Septoglossum constrictum</i>	82 (6.3)	12 (2.1)	<b>0.002</b>
<i>Diversispora epigaea</i>	10 (0.8)	3 (1.5)	<b>0.004</b>
<i>Paraglossum occultum</i>	14 (1.1)	2 (0.4)	<b>0.005</b>
<i>Palaeospora spaineae</i>	6 (0.4)	2 (0.4)	<b>0.008</b>
<i>Rhizoglossum irregulare</i>	30 (2.3)	14 (2.3)	<b>0.009</b>
<i>Dominikia compressa</i>	39 (3.0)	8 (1.4)	<b>0.011</b>
<i>Glomus badium</i>	13 (1.0)	1 (0.1)	<b>0.017</b>
<i>Funneliformis fragilistratus</i>	13 (1.0)	5 (0.8)	<b>0.017</b>
<i>Sclerocystis sinuosa</i>	399 (31.3)	140 (23.9)	<b>0.021</b>
<i>Diversispora celata</i>	10 (0.8)	1 (0.1)	<b>0.021</b>
<i>Dominikia aurea</i>	91 (7.1)	3 (0.5)	<b>0.026</b>
<i>Funneliformis geosporus</i>	131 (10.2)	73 (10.4)	<b>0.029</b>
<i>Dominikia bernensis</i>	139 (10.8)	36 (6.2)	<b>0.045</b>
<i>Sclerocystis</i> sp.	33 (2.6)	9 (1.5)	0.054
<i>Paraglossum turpe</i>	7 (0.6)	3 (0.5)	0.071
<i>Rhizoglossum intraradices</i>	10 (0.8)	6 (1.0)	0.089
<b>Exclusively found in the no-till vineyard</b>			
<i>Glomus macrocarpum</i>	45 (3.5)	-	
<i>Rhizoglossum aggregatum</i>	15 (1.2)	-	
<i>Glomus microcarpum</i>	6 (0.5)	-	
<i>Acaulospora longula</i>	3 (0.2)	-	
<i>Claroideoglossum etunicatum</i>	3 (0.2)	-	
<i>Ambispora gerdemannii</i>	2 (0.2)	-	
<i>Glomus heterosporum</i>	2 (0.2)	-	
<i>Cetraspora armeniaca</i>	1 (0.1)	-	
<i>Acaulospora laevis</i>	1 (0.1)	-	
<i>Dominikia</i> sp. BR11	1 (0.1)	-	
Total number of spores identified 100g <sup>-1</sup> soil	1286	585	
Total AMF species richness at site	34	24	

Average and standard deviation of five plots per treatment; p-values <0.05 show significant differences between the two vineyards according to student t-test.

tal only seven spores of *Acaulospora laevis*, *A. longula*, *Ambispora gerdemannii* and *Cetraspora armeniaca* were detected), which is in accordance with findings of SCHREINER and MIHARA (2009). All these fungal taxa are known to react negatively not only on soil disturbance, but also to high fertilization levels (OEHL et al., 2010, 2011, 2017; MAURER et al., 2014). The high nutrient levels in the vineyards thus can explain, why no high spore abundances and species richness of these specific AMF genera and families were found, although these AMF taxa are frequent members of Central European soils with pH 5.0-7.0 (BŁASZKOWSKI, 1993; OEHL et al., 2004, 2010; MAURER et al., 2014). Extensive future studies in Central European vineyards have to prove if our assumptions are valid. The situation might be quite different even within growing areas, such as in Palatinate, as grapevine areas are often geologically quite diverse and have a very wide range of soil types respective different 'terroir' (VAN LEEUVEN and SEGUIN, 2006; KOCH and OEHL, 2018), and thus, may naturally have quite diverse AMF communities (OEHL et al., 2010, 2017).

Of the 34 AMF species detected in the present study, six species were found in similar spore abundancies in both vineyards, among them *Claroideoglossum claroideum* and *Cl. luteum*, *Rhizoglossum fasciculatum* and *Rh. invermaium*, and *Funneliformis mosseae*. Two AMF species were more frequent in the cultivated inter-rows than in the no-tillage inter-rows (*Glomus diaphanum* and *Archaeospora trappei*), while 16 species were, more or less clearly, more abundant in the no-tillage inter-rows than in the cultivated inter-rows (e.g. *Septoglossum constrictum*, *Glomus badium*, *Sclerocystis sinuosa*, *Diversispora celata* and *Dominikia compressa*). The lasting ten species were exclusively detected in the no-tillage inter-rows (e.g. *Glomus macrocarpum*), but some of them with a few spores only (e.g. *Cetraspora armeniaca* and *Acaulospora laevis*). These results again fit well with the former results from tilled and of non-tilled croplands, or vineyard in the areas of Baden and Rheinhessen in Germany (e.g. OEHL et al., 2005a, 2005b, 2010; MAURER et al., 2014; WETZEL et al., 2014). However, we can only assume, according to Smith and Read (2008) and all other literature about AM fungi and their symbiosis, that all the AMF species detected in our inter-rows are also symbionts of "Pinot Gris" plants themselves. Only detail study in the greenhouse or molecular root analyses can elucidate the AMF compositions within the grape roots (e.g. SCHREINER and MIHALA, 2009; LUMINI et al., 2010; LIKAR et al., 2013), when compared to those in the roots of the different green cover plants.

### Conclusions

Arbuscular mycorrhizal research has got a major importance during the last decades, but the majority of the AM fungal species worldwide have not yet been discovered. Moreover, we are still far from understanding and using the potential of the beneficial effects of AMF for agronomical important crops. We do not know what even the most intensively investigated AMF species can provide for the plants, soils, entire habitats and complex ecosystems. It has become a challenging task to figure out, how we can use these fungi for agricultural purposes or environmental benefits. We assume that AMF and their diversity might be important for high quality grapevine and wine production, especially in regions of scarce rainfall, due to their capacity to deliver nutrients and water to the plants, and to protect soils against erosion and compaction during and after heavy rains, respectively. For their potential future use in vineyards, it is a precondition to know the effects of specific management practices and cultivation techniques on the development and biodiversity of these AM fungi, and to know how we can maximize the ecosystem services, which they provide in our soil-plant systems, such as for grapevine and wine production. In vineyards under semi-arid conditions, no-till temporary and permanent plant cover in the grapevine inter-rows is a viable alternative to traditional soil tillage systems, but one of the challenges will be

to establish and sustain beneficial plant and microbial communities that deal with the periodical water scarcity and do not compete but promote water and nutrient uptake, and thus grapevine growth and quality.

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