Agriculture, Ecosystems and Environment 248 (2017) 144–152

Contents lists available at ScienceDirect

Agriculture, Ecosystems and Environment

journal homepage: www.elsevier.com/locate/agee

High soil phosphorus levels overrule the potential benefits of organic farming on arbuscular mycorrhizal diversity in northern vineyards

Maarten Van Geel[a,*], Erik Verbruggen[b], Matthias De Beenhouwer[a], Geurt van Rennes[c], Bart Lievens[d], Olivier Honnay[a]

[a] Plant Conservation and Population Biology, Department of Biology, KU Leuven, Kasteelpark Arenberg 31, B-3001 Heverlee, Belgium
[b] Department of Plant and Vegetation Ecology, University of Antwerp, Universiteitsplein 1, B-2610 Antwerpen, Belgium
[c] BeNeVit, Maastrichtersteenweg 79, 3500 Hasselt, Belgium
[d] Laboratory for Process and Bioinspirational Management (PME & BIM), Department of Microbial and Molecular Systems (M2S), KU Leuven, Campus De Nayer, B-2860 Sint-Katelijne Waver, Belgium

ARTICLE INFO

Keywords:
AMF
Biodiversity
Bordeaux mixture
Copper
Eutrophication
Vitis vinifera

ABSTRACT

Organic farming is a key approach to reconcile food production, biodiversity conservation and environmental sustainability. Due to reduced inputs of agrochemicals, the success of organic farming is heavily dependent on the ecosystem services provided by the soil microbial community, and in particular by arbuscular mycorrhizal fungi (AMF). Numerous studies have already shown that also grapevines (*Vitis vinifera*) depend on AMF for normal growth and development. To what extent organic agriculture benefits the AMF communities on vines at regional scales, however, is still poorly understood. Here, we first quantified the relative importance of organic management, soil chemical characteristics, and geography on vineyard AMF diversity and community composition. Second, we tested whether soil nutrients fundamentally change the host-AMF community dynamics through changing universality of dissimilarity overlap curves. To identify AMF communities, we used high-throughput pyrosequencing on 170 root samples from grapevines originating from 18 conventionally and 16 organically managed Belgian and Dutch vineyards. We found no differences in AMF diversity between conventionally and organically managed vineyards. Soil phosphorus content and soil acidity, however, was strongly negatively associated with AMF diversity. Together with management type (organic vs. conventional), these two soil variables did also explain most of the variation in AMF community composition. The observed accumulation of soil copper, used to control fungal diseases, especially in organically managed vineyards, did not affect AMF communities. We observed, however, that copper concentration in the soil increased with vineyard age, indicating copper accumulation in the soil over time. AMF communities showed a regularity in interactions among taxa and their host. Under high soil P availability, however, interactions became more irregular. The potential benefits of organic vineyard management in terms of a high diversity of AMF are highly compromised by elevated soil phosphorus levels which may jeopardize the role of these symbionts in improving plant health and soil fertility. Decreasing nutrient inputs, even organic, is a key step in developing diverse AMF communities in vineyards.

1. Introduction

The use of high-yielding crop varieties, chemical fertilizers and pesticides in combination with mechanization have dramatically increased worldwide agricultural production since the 1950s. At the same time, the application of agrochemicals has resulted in the eutrophication and contamination of soil and water, and has severely simplified agricultural ecosystems in terms of their species richness (Tilman et al., 2001; Geiger et al., 2010). As there is compelling evidence that biodiversity benefits the provision of a range of ecosystem services (Cardinale et al., 2012), this simplification can be expected to jeopardize the ecosystem services delivered by agricultural ecosystems. It is in this context that organic farming has been proposed as a key approach to reconcile food production, biodiversity conservation and environmental sustainability. As organic farming practices exclude the use of chemical fertilizers and pesticides, it heavily relies on natural biological processes for both the nutrient supply and the protection of the crops grown (Tittonell, 2014). Therefore, the soil microbial

* Corresponding author.
E-mail address: maarten.vangeel@bio.kuleuven.be (M. Van Geel).

http://dx.doi.org/10.1016/j.agee.2017.07.017
Received 14 December 2016; Received in revised form 24 April 2017; Accepted 13 July 2017

community is vital for the success of organic farming and for the functioning of agroecosystems in general (Bowles et al., 2016). Particularly arbuscular mycorrhizal fungi (AMF) are important components of the soil microbial community in agricultural ecosystems as they contribute to plant health and soil fertility (Rillig et al., 2016). As compared to other crop species, the AMF communities that associate with grapevine (*Vitis vinifera*) may be of even greater importance because they may contribute to the microbial terroir of vines, providing distinct characteristics to the grapes and the wine produced (Trouvelot et al., 2015).

AMF are key components in agricultural ecosystems and form a symbiosis with the majority of the land plants. In return for plant photosynthates, AMF provide a range of benefits to the host through their extraradical hyphal network, which acts as a living interface between the roots and the soil. Numerous studies have already shown that also grapevines depend on AMF for normal growth and development (reviewed in Schreiner, 2005). AMF mainly increase phosphorus (P) and nitrogen (N) uptake by grapevines, but increased uptake of other nutrients, such as zinc, copper, potassium and calcium have been reported as well (Schreiner, 2005). AMF can also enhance grapevine tolerance to abiotic stress conditions, such as drought (Valentine et al., 2006), salinity (Belew et al., 2010) or heavy metals (Karagiannidis and Nikolaou, 2000). These effects are thought to partly stem from systemic plant responses that are associated with marked changes in secondary metabolite composition of tissues (Doehlemann et al., 2014). Furthermore, AMF can protect grapevine from soil-borne pathogens (Hao et al., 2012) and stabilize the soil through entangling soil particles with their hyphae (Rillig and Mummey, 2006). Given both their potential importance for developing a microbial terroir and the reported beneficial effects of AMF on grapevine, it is crucial to understand how organic vineyard management practices and local soil characteristics can influence AMF communities in the roots of grapevine, across larger geographical scales.

Organic agriculture has been shown to increase the diversity of AMF in many crop species (e.g. Verbruggen et al., 2010). How organic agriculture affects AMF diversity in grapevine, however, is hardly known (only from small scaled studies using microscopic analysis or genetic fingerprinting techniques, e.g. Balestrini et al., 2010; Likar et al., 2013). Furthermore, high fertilizer inputs have widely been recognized to negatively affect AMF abundance in a large variety of crop and plant species (Jansa et al., 2009). Also in grapevine, it has been shown that high soil P levels reduce root colonization of specific AMF taxa (Karagiannidis and Nikolaou, 1999), whereas N fertilization suppressed colonization and sporulation of specific AMF taxa (Karagiannidis et al., 2007). How entire AMF communities in grapevine change with increasing soil P or N levels, and to what extent the AMF community shows signs of an altered dynamic in response to high nutrient levels, is still poorly known. Since the end of the nineteenth century, copper sulfate (Bordeaux mixture) has been used in vineyards to control vine fungal diseases, such as Downy mildew (*Plasmopara viticola*). Copper based fungicides are currently also the only allowed way to control plant pathogenic fungi in organically managed vineyards. The practice has resulted in a widespread accumulation of copper in the soil. Whereas normal background concentrations of copper range from 5 to 30 mg kg^{-1}, copper concentrations ranging from 100 up to 1500 mg kg^{-1} have been measured in European vineyards with a long history of copper-based fungicide use (Flores-Vélez et al., 1996). High soil copper concentrations have been shown to negatively affect a wide range of soil biota in agricultural ecosystems (e.g. Van Zwieten et al., 2004), but to what extent copper affects AMF communities is still unknown.

Recent advances in microbiome bioinformatics have greatly increased the toolbox at our disposal to test for patterns in metagenomic data that can inform us on underlying community ecological processes. One such tool is the recently formulated and successfully applied dissimilarity overlap curve (DOC) (Bashan et al., 2016), which analyzes the relationship between overlap in community composition and the dissimilarity in relative abundances of all taxa. A negative slope in the high-overlap region of the DOC indicates a regularity in interactions among taxa and their host, *i.e.* 'universality'. In contrast, the absence of this relationship indicates that interactions are irregular, or 'individual' based. Applying DOC analysis to AMF communities interacting with grapevines informs us on the universality of hosts interacting with their AMF symbionts, which has been found to commonly occur in other host-microbe interactions such as those between humans and gut and mouth microbiomes that display pronounced universal dynamics (Bashan et al., 2016). This further allows us to assess whether the host-AMF community interaction is resistant to the disturbance imposed by high nutrient levels in terms of its stability across individual grapevines.

Here, we applied high-throughput pyrosequencing on 170 root samples from grapevines originating from 18 conventionally and 16 organically managed vineyards in northern Belgium and the southern part of The Netherlands and aimed to (i) evaluate the benefits of organic farming on the AMF communities present; (ii) quantify the relative importance of soil chemical variables, including nutrients and copper, and management type (conventional *vs.* organic) on AMF diversity and community composition; and (iii) test whether soil nutrients fundamentally change the host-AMF interaction through changing universality of dissimilarity overlap curves.

2. Materials and methods

2.1. Study sites and sampling

The study was conducted in Flanders, the northern part of Belgium, and the most southern part of the Netherlands. Annual average precipitation is 785 mm and average annual temperature is 9.8 °C. A total of 34 vineyards were examined within this study (average distance between vineyards was 87.9 km, minimal 1 km, maximal 223 km) (Supporting information Fig. S1 and Table S1). Three vineyards were located in the Netherlands, just across the Flemish border (Vineyard 30, 31 and 32) (Supporting information Fig. S1). A stratified random sampling design, stratified by the type of management, was used. We sampled 18 conventionally and 16 organically managed vineyards (Supporting information Fig. S1). In the organic vineyards, no chemical fertilizers, or pesticides were used since transformation to organic management. However, organic fertilizers and small amounts of copper-based fungicides to control Downy mildew (*Plasmopara viticola*) were allowed. Planting density or plant age did not differ between both types of vineyards. All grapevines were grafted on SO4 rootstocks, a frequently used rootstock for commercial grapevine production. In October 2015, roots from five randomly chosen grapevines per vineyard were excavated. Root samples were collected at three random locations around each grapevine and were pooled afterwards to obtain one pooled root sample per grapevine. Especially fine roots were collected, as these are known to contain AMF. A soil sample for chemical analysis was also collected near each sampled individual. Root samples were stored at 4 °C until further analysis. Soil samples were stored at 4 °C for maximum one week to prevent nitrogen loss. Preservation at 4 °C slows down microbial mediated denitrification to the extent that virtually no nitrogen is lost in the sample in the time span of one week. In total, 170 root and 170 soil samples across the 34 vineyards were obtained.

2.2. Soil chemical analysis

Soil pH was quantified using a pH probe in a 1:10 soil/water mixture. As a measure of the plant-available N content of the soil, ammonium and nitrate availability were quantified by shaking 10 g of soil in 200 ml of 1 M potassium chloride solution for one hour. Extracts were analyzed colorimetrically using a segmented flow auto analyzer (Skalar, Breda, the Netherlands). As a measure of the plant-available P content