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Ecological and conventional viticulture gives rise to distinct fungal and bacterial microbial communities in vineyard soils

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ABSTRACT

The new ecological approach to viticulture emphasises ecologically sound grape production and recognizes the importance of grapevine (*Vitis vinifera*) interactions with soil microbial communities. Due to different viticulture measures, distinctly different microbial communities can form, which affects the potentially beneficial interactions with the grapevines. Therefore, the objective of the present study was to identify differences in the community structures of fungal and bacterial soil communities on a landscape scale, and to relate these to the type and duration of soil management, and to within vineyard habitats. Soil fungal and bacterial communities were screened in vineyards with different soil management (ecological, conventional) and ages (3, 10, 35 years). In each vineyard, 35 rhizosphere soil samples were acquired from beneath the grapevines and between the rows, using a grid covering of 240 m^2 per vineyard. Automated ribosomal intergenic spacer analysis of the microbial soil samples was carried out, with the microbial lifeforms compared among the vineyards (bacteria *vs.* fungi). There was high variability in the local microbial diversity for all of the sampled plots, with significant differences among the vineyards under ecological and conventional soil management. In comparison to fungi, soil management and age of vineyard had significantly greater impact on bacteria. Microbial communities showed no general overlap in diversity spots or simultaneous changes in community composition along the sampling grids. These results suggest that the microbial communities reflect the influences of highly localised biogeographic factors and vineyard management, with the most profound effects observed after deep tillage. Furthermore, fungal and bacterial communities forming in the vineyard soils appear to be influenced by different sets of environmental factors.

1. Introduction

The new ecological approach to viticulture with emphasis on ecologically sound grape (*Vitis vinifera*) production views grapevines as part of a complex agroecosystem where many organisms co-exist and interact. In particular, this approach recognizes the importance of interactions between the microbial communities and the plants (Likar et al., 2015; Regvar et al., 2012), as these influence the growth, physiology and yield of the grapevines.

In conventional viticulture, severe negative effects on soil microbial communities can be caused by fungicide application (Sigler and Turco, 2002), by acidification of the soil due to fertiliser input (O'Donnell et al., 2001; Muñoz-Leoz et al., 2012), and by tillage practices (Mijangos et al., 2006). Pesticide application can also significantly affect microbial communities, including beneficial groups like mycorrhizal fungi (Chi-Chu, 2010; Menge et al., 1978). These practices can thus change the interactions between grapevines and microorganisms.

Synthetic fungicides are the main pesticides used in conventional viticulture, while copper-based fungicides are the only effective methods permitted for ecological viticulture. However, prolonged use of copper can also have profound effects on microbial communities, as copper accumulates within the topsoil following fungicide application (Pietrzak and McPhail, 2004; Rusjan et al., 2007). Copper also becomes mobile at soil pH from 5.5 to 6.5, and thus more available to organisms, which can create stress for microorganisms and affect their enzyme activities (Dell'Amico et al., 2008; Hinojosa et al., 2010; White, 2009).

In addition, soil management practices, like tillage (Mathew et al., 2012) and fertilising (Lazcano et al., 2013), and weed

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communities that grow in vineyards (Radić et al., 2014) also influence the physical and chemical characteristics of the soil. Similarly, the diversity of the permanent green cover between vineyard rows, in particular, can strongly influence soil microbial diversity and function (Lange et al., 2015).

In contrast, the low-input measures of environmentally sound, or ecological, viticulture can provide better conditions to support higher diversity of beneficial microorganisms, such as mycorrhizal fungi (Likar et al., 2013; Radić et al., 2012, 2014). These measures can avoid selection of taxa that tolerate high nutrient levels (Hijri et al., 2006). This is especially important for plants such as grapevines, as these are characterised by low root densities and can have relatively coarse fine roots, which indicates the need for strong dependence on interactions with beneficial root endophytes (Biricolti et al., 1997; Karagiannidis and Nikolaou, 1999; Linderman and Davis, 2001).

Although the new ecological approach to viticulture recognizes the importance of grapevine interactions with soil microbial communities, there remains little knowledge available of the effects that different viticultural techniques can have on the formation of soil microbial communities. Information on the process of formation of distinct and functional microbial communities in production vineyards is therefore a key element in an understanding of their possible beneficial interactions with the grapevines and with grape production. Therefore the objective of the present study was to identify differences in the community structures of fungal and bacterial soil communities on a landscape scale, and to relate these to the type and duration of the soil management, and the within-vineyard habitats.

We hypothesised that differences in these viticulture measures will give rise to distinctly different and potentially beneficial microbial communities that will further differentiate based on the length of the ecological management. Furthermore, we wanted to evaluate the degree of similarity in the responses of the bacteria and fungi to the environmental conditions in the individual vineyards, and thus we analysed the communities using a grid-based pattern.

2. Materials and methods

2.1. Study area

Samples were taken from ecological vineyards and a conventional vineyard located near Šempas, Vipavska dolina, Slovenia (N 45° 55.614, E13° 44.568). The site is at an altitude of approximately 90 m above sea level, and has a mean annual precipitation of 1500 mm, which is mostly distributed in the spring and autumn. The mean annual temperature was 12 °C (range, 8–18 °C), with 90 sunny days per year.

This winegrowing region is characterised by strong winds (the 'bora') that can reach peak velocities that exceed 130 km h^{-1}, which are thus linked to significant surface-soil erosion (Komac and Zorn, 2005; Zorn, 2008). The area bedrock was classified as Cretaceous platform carbonate rock, limestone–dolomite non-clastic siliceous sedimentary rock, according to the geological composition (Geological Survey of Slovenia, http://kalcedon.geo-zs.si/website/PTGK/viewer.htm). The soil was Eutric brown soil, typic and calcaric on flysch (Eutric, Calcaric Cambisols) (FAO, 1974, 1988).

In the present study, soil samples were collected from four vineyards: vineyard S3, with 3 years of ecological management; vineyard S10, with 10 years of ecological management; vineyard S35, with 35 years of ecological management; and vineyard CV35, with 35 years of conventional management (Supplementary materials Figs. S1, S2; Table 1). The grapevines in the vineyards under ecological management are integrated into the biological/organic production, where the practices set out in "Rules on organic production and processing of agricultural products and/or foods" (Official Gazette of RS, 2001, 2003, 2006) should be taken into considerations. The allowed phytochemical agents in viticulture are in general copper hydroxide ($Cu(OH)_2$) and copper oxychloride ($3Cu(OH)_2 \times CuCl_2$) against downy mildew (*Plasmopara viticola*), at a maximum of 6 kg of pure copper ha^{-1} $year^{-1}$. Only sulphur in solid forms (powder) can be used against powdery mildew (*Uncinula necator*). The concentrations of the phytochemicals for spraying are prepared according to the instructions of their producers, and although the number of applications in any vineyard will vary, in general they do not exceed five to seven applications per grapevine growing season. The soil management consists of shallow tillage in the spring only, and during the summer, autumn and winter, the vineyard should be permanently green and the use of herbicides is forbidden.

All of these vineyards had permanent green cover, as a mixture of annuals and herbaceous perennials. Vineyard S3 had undergone recent replanting of the whole vineyard, and hence a different successional and less-dense plant cover was observed. The plant cover was estimated for five 1-m^2 plots per vineyard, and was generally 90% to 100% cover (vineyards S10, S35, CV35), but only 10% to 20% for vineyard S3. The maximum distance between these vineyards was 335 m (between S3 and S10).

Table 1
Soil physical and chemical characteristics, plant cover, and extracted DNA purity for the sampling plots inside the individual vineyard.

Parameter	Vineyard[a]			
	S3	S10	S35	CV35
Plant-available Cu (mg kg^{-1})	0.95 ± 0.11	3.09 ± 0.18	1.96 ± 0.19	2.27 ± 0.16
Plant-available P (mg 100 g^{-1})	0.01 ± 0.001	0.48 ± 0.008	0.15 ± 0.04	0.06 ± 0.005
Organic matter (%)	1.20 ± 0.05	2.70 ± 0.12	2.79 ± 0.08	4.71 ± 0.13
pH	7.35 ± 0.04	7.48 ± 0.02	6.91 ± 0.06	5.85 ± 0.06
Potential acidity	6.75 ± 0.05	6.94 ± 0.01	6.17 ± 0.08	4.91 ± 0.06
Soil water content (%)	6.74 ± 0.36	4.57 ± 0.09	6.65 ± 2.04	3.98 ± 0.1
Soil texture	sandy loam	sandy loam	silt loam	loam
Plant cover (%)	10–20	90–100	90–100	90–100
Microbial activity (mg fluorescein diacetate hydrolyzed g $soil^{-1}$ h^{-1})	1.03 ± 0.11	2.36 ± 0.14	1.95 ± 0.12	1.97 ± 0.11
DNA absorbance ratio at 260/280 nm	1.1 ± 0.12	1.79 ± 0.07	1.95 ± 0.03	1.89 ± 0.07
DNA absorbance ratio at 260/230 nm	0.36 ± 0.12	1.26 ± 0.06	1.65 ± 0.08	1.91 ± 0.1

Note: Data are means ± standard error (n = 5 for plant cover estimation and n = 35 for other parameters).
[a] S3 – 3 years of ecological management; S10 – 10 years of ecological management; S35 – 35 years of ecological management; CV35 – 35 years of conventional management.