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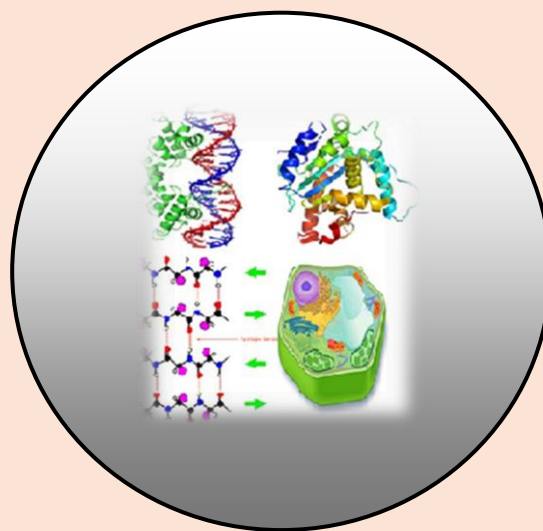
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RESEARCH PAPER

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Soil Microbial Indicators within Rotations and Tillage Systems in Grapes Cultivation

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ABSTRACT

*Medicinal properties attributed to the Grape (benefits) Leaves have venotonic, vasoprotective, astringent and diuretic effects. The fruits are vitaminics, tonics, anticancer, hepatoprotective, promote hair growth and prevent ischemic processes. Cover crops are increasingly adopted in viticulture to enhance soil quality and balance the vegetative and reproductive growth of vines. Nevertheless, this sustainable practice has been only recently used for table grape viticulture, with results often contrasting. The aim of this study was to assess the effect of a fescue (*Festuca arundinacea* Schreb.) cover crop on soil quality, yield, and grape qualitative parameters. Soil organic carbon (C), total nitrogen (N), microbial biomass C (MBC), β -glucosidase (BGLU) and alkaline phosphomonoesterase (APME) activities were assessed during different growing winter season. The trend of soil chemical and microbiological properties was jointly influenced by the soil management system, growing season and phenological stage. Compared to conventional tillage, cover crops increased, on average, soil organic C, total N, MBC, BGLU and APME by 136%, 93%, 112%, 100% and 62%, respectively. Slight or no effects of cover crops were observed on grape quality and yield, except for 2022. This study reveals that cover crops strongly enhance soil quality in the short-term, with potential advantages for grape production in the long-term.*

Keywords: *Vitis vinifera*; Soil quality; Soil microbial biomass; β -glucosidase; Alkaline phosphomonoesterase and Grape medicinal properties.

INTRODUCTION

Grapevine (*Vitis vinifera* L.) is one of the main fruit crops cultivated all over the world. Agricultural management practices influence soil microbial communities, creating niche environments that favor certain microbes [Kheyroodin and Antoun, 2011]. Management practices can include crop rotation, tillage, N fertilization, cover cropping, etc.

By selecting management practices or combining them, the soil environment is altered, as are essential soil processes. These can include residue decomposition, nutrient and water cycling, aeration and gaseous interactions, development of soil aggregates, soil organic matter (SOM) dynamics, and biodiversity measures [Kheyrodin et al., 2012, Kheyrodin, 2014]. Crop rotation is a common management practice with benefits that include pest and disease control and yield improvement and stabilization [Kheyrodin and Ghazvinian, 2012, Kheyrodin, 2009]. Tillage is another tool used to improve yields by creating a more favorable environment for cash crop growth. In systems of high organic matter, tillage ensures a clean seedbed for early growth by reducing compaction, improving aeration, increasing soil temperature, and removing weed competition [Kheyrodin and Ghazvinian, 2012, Kheyrodin, 2016, Kheyrodin, 2014]. Lastly, N fertilization is a common practice used to enhance yields, and that influx of previously scarce N reshapes potential N dynamics controlled by soil microbial communities [Kheyrodin and Ghazvinian, 2013]. Given the benefits of crop rotation, tillage, and N fertilization on crop yields, their implementation is widespread, which affects the soil microbial community. Crop rotation and fertilization alter the quantity and quality of crop residues, root exudates, and subsequent rhizodeposits [Kheyrodin and Ghazvinian, 2012, Kheyrodin and Antoun, 2011]. Results from a meta-analysis by Ouyang et al. [2014] showed that crop rotation and soil pH influenced N cycling by changing the ammonia-oxidizing bacteria (AOB) and archaea (AOA) community dynamics, as well as denitrifiers. Crop rotation increased AOB levels compared to monocultures; AOA was unaffected. However, neutrophilic soil conditions led to an increase in both AOA and AOB. Furthermore, N fertilization increased the abundance of AOA, AOB, and denitrifiers [Kheyrodin and Ghazvinian, 2012]. In a remarkably long-term study on the Morrow Plots in Urbana, IL, treatments of crop rotation and N fertilization have been in place since 1876; shifts in microbial functions related to substrate utilization were affected by fertilizer treatments more than crop rotation given the chronic nutrient limitations and changes in soil chemistry [Kheyrodin and Antoun, 2012]. Smith et al. [2016] reported that crop type and tillage altered species composition, but not quantity and diversity metrics from Indiana corn (*Zea mays* L.)–soybean [*Glycine max* (L.) Merr.] rotations. Compared to crop rotation, tillage had a larger effect on nutrient levels, which was a better predictor for microbial community composition [Smith et al. 2016]. The effects of tillage on the soil microbial community include mechanically disrupting growth and distribution, destroying soil aggregates, reducing soil moisture, increasing soil temperature, and degrading soil organic matter (SOM) [Kheyrodin, H and , K Ghazvinian. 2015, Kheyrodin, and Antoun, 2008]. In a global meta-analysis looking at the effects of tillage, Zuber and Villamil observed that NT systems have greater microbial biomass and enzymatic activities. Furthermore, de Graaff et al., also using a meta-analytic approach, showed that tillage decreased bacterial biodiversity, however, it did not affect fungi. Previously when technology was a limiting factor, using broad inference measurements was the best available technique for explaining how the soil microbiome responds to management factors. However, new metagenomic approaches better characterize the microbial community composition and function and its relationship with soil properties and agronomics. Diversity and richness metrics represent the variability within a single sample (α -diversity) and among communities (β -diversity). Using quantitative polymerase chain reaction (qPCR), functional microbial genes, such as *nirK*, which is involved in denitrification, are analyzed for treatment effects.

Lastly, using primers for each major taxonomic group (bacteria, fungi, and archaea, PCR amplification produces a vast pool of amplicons. From that pool of hundreds to thousands of individual amplicons, high throughput sequencing with Illumina yields a deep inventory of amplicon sequence variants (ASVs), from where indicator microbes can be selected and characterized. Indicator microbes usually refer to an ASV that explains variability in a dataset. Studies on indicator microbes have shown that organic matter inputs and pH alter the cycling of N and C, resulting in significant changes in soil biological properties. Given the complexity of using metagenomics to identify indicator microbes, field studies are scarce, especially from a long-term setting. A few long-term studies (15–130 years) have determined indicator microbes from typical cropping systems [Kheyrodin and Antoun, 2012], though none have analyzed crop rotation and tillage simultaneously. As these are the most common tools used by growers to improve yields, a thorough investigation of these indicator microbes is necessary.

We hypothesized that our treatments of continuous corn and soybean would show contrasting effects on microbial taxa, with rotated corn-soybean having intermediate results, not different from either monoculture. We also hypothesized that AOB and fungi would have elevated abundances in the continuous corn treatment, with AOA increasing in the continuous soybean treatment. Therefore, the objective of this investigation was to identify microbial taxa that were responsive to crop rotation and tillage from a long-term, stable trial (20+ years). The results will add valuable primary information on how the soil microorganisms shift in response to common agricultural management practices.

MATERIALS AND METHODS

Experimental Site Description and Management Practices

The experiment was conducted at the Semnan University, Agricultural Research and Demonstration Center (35.2256° N, 54.4342° E). The study was established in 2022, and a complete description of the site can be found in Kheyrodin and Antoun 2911. Briefly, soils were comprised of highly fertile silty clay loam and silt loam soil series (Muscatune 43%, Sable 40%, and Osco 17%) [Kheyrodin and Antoun, 2011]. The study was designed in a split-plot arrangement of 4 rotation levels and 2 tillage levels in a randomized complete block design with 4 replications (blocks). The main plots (22 m long by 12 m wide) were crop rotation treatments, which consisted of continuous (CCC).

Soils were characterized for the following physicochemical properties: texture [Kheyrodin and Antoun, 2008], pH (both in H₂O and in a CaCl₂ solution, using in both cases a soil/solution ratio of 1:2.5, m/v), electrical conductivity (EC), organic C content (Walkley–Black method), total N content (Kjeldahl method), total and active CaCO₃ [Kheyrodin, 2013], and available P (Olsen method). All the chemical analyses, except for texture and CaCO₃ determination, were carried out following the standard procedures for soil analyses reported by Kheyrodin [Kheyrodin and Antoun, 2011].

Soil microbial biomass C (MBC) was measured through the fumigation-incubation-extraction method [Kheyrodin and Antoun, 2011, Kheyrodin, 2014]. Each field-moist soil sample (containing 50 g oven-dried soil) was divided in half: a non-fumigated half (control) was immediately extracted with 100 mL 0.5 M K₂SO₄ for 30 min, whereas the remaining part was fumigated with chloroform in the dark, for 24 h at 25 °C.

After fumigation, CHCl_3 was removed and soil samples were extracted as indicated for the control. Organic C in the soil extracts was measured using the Springer–Klee method. The β -glucosidase (BGLU) activity of soils was determined according to Kheyrodin and Antoun [Kheyrodin and Ghazvinian, 2015], measuring spectrophotometrically (at 400 nm wavelength) the amount of p-nitrophenol (PNP) released after 1 h of soil incubation with a p-nitrophenol- β -D glucoside solution, at 37 °C. The alkaline phosphomonoesterase (APME) activity was determined following the procedure described by Kheyrodin and Antoun [Kheyrodin and Ghazvinian, 2015]. Briefly, soil was added with a buffered (pH 11) p-nitrophenyl phosphate solution and, after 1 h of incubation at 37 °C, the amount of PNP released as a result of the hydrolytic activity was measured spectrophotometrically at 400 nm wavelength.

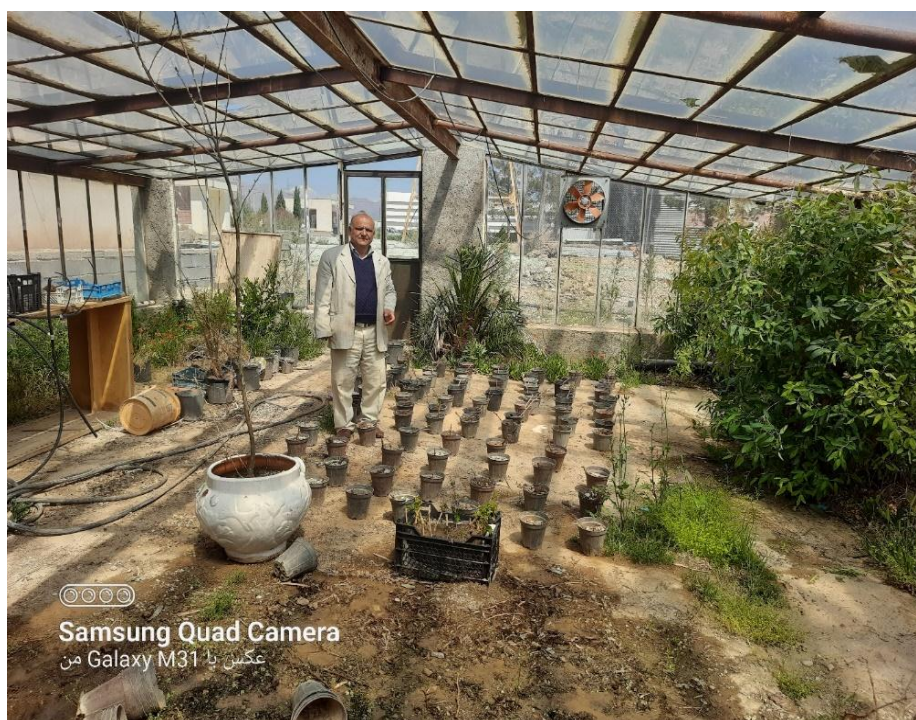


Figure 1. The experiment was conducted at the Semnan University Agricultural Research and Demonstration.

RESULTS AND DISCUSSION

The results showing in fig 2, 3. The great abundance and diversity of microorganisms in soil have high metabolic potentials. Since microorganisms are generally growth-limited in soils, they may poorly exploit their capabilities. The present study demonstrates that the fescue cover crop strongly improves, in the short-term (three years), the soil chemical and microbiological properties of a table grape vineyard. In particular, the average increase of 136% of organic C in the cover crop treatment is impressive, considering the very low initial organic C content of the soil (2.6 g kg^{-1}). This enhanced C sequestration, along with the increased total N content and root biomass (due to the absence of tillage passes), stimulates the soil microbial growth.

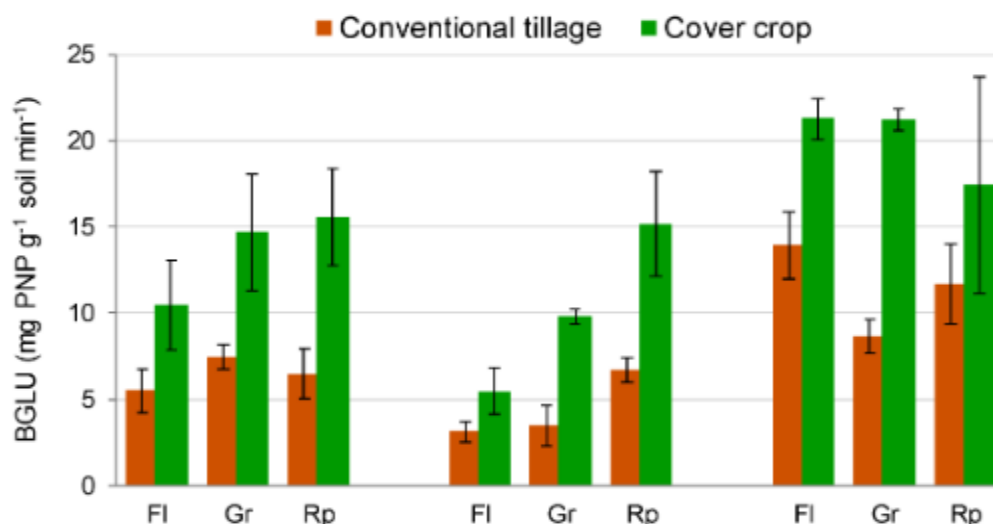


Figure 2. The trend of β -glucosidase activity (BGLU) in soil subjected to conventional tillage or cover crop during flowering (FI), fruit growth (Gr) and ripening (Rp) in the three years of experimentation. The bar on each column indicates the standard deviation ($n = 3$).

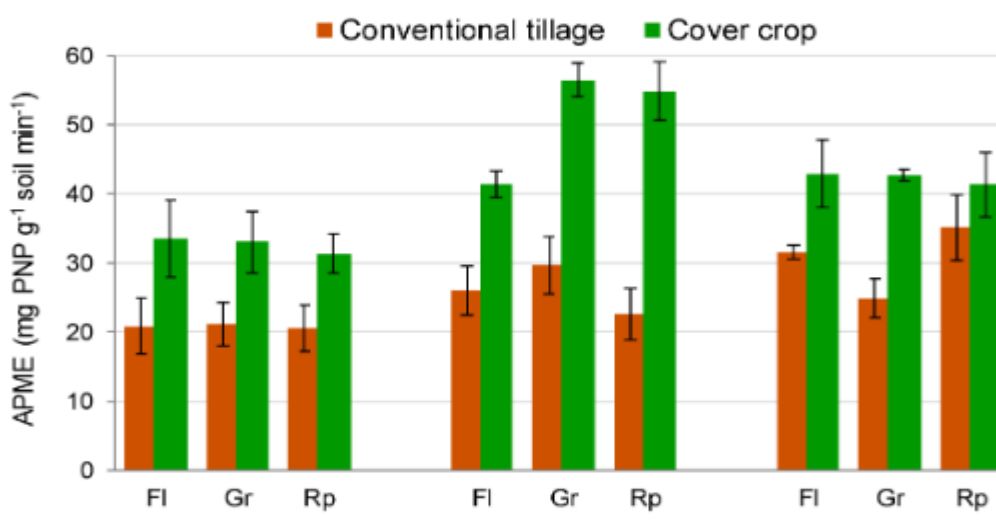


Figure 3. The trend of alkaline phosphomonoesterase activity (APME) in soil subjected to conventional tillage or cover crop during flowering (FI), fruit growth (Gr) and ripening (Rp) in the three years of experimentation.

In this review Too we conducted that grapes are a rich source of bioactive molecules including phenolic acids, flavonoids, anthocyanins, stilbenes, and lipids. These are the compounds which contribute to the health benefits of grape and grape-derived products. They possess antioxidant, antimicrobial, anti-inflammatory, and anti-carcinogenic activities and have wide applications in food and nutraceutical industries. Use of grape extracts rich in these bioactive compounds are linked to reduced incidence of cardiovascular disease and its major risk factors including hypertension (high blood pressure); a clinical condition associated with high mortality worldwide. Therefore, considerable attention has been given to grape-based products to alleviate and treat hypertension.

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