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Fungal communities are differentially affected by conventional and biodynamic agricultural management approaches in vineyard ecosystems

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ABSTRACT

There is increased need to identify sustainable agricultural methods which avoid environmental degradation. Previous studies have focused on the effect of specific agricultural interventions on large organisms, but we have fewer data evaluating how microbes, which are key components of ecosystems, might be affected. Additionally, previous studies have been constrained as they only examined one habitat in an ecosystem and have not gone on to evaluate the effect of agricultural approach on harvested crops. Here we take an ecosystems approach and evaluate the net effect of conventional versus biodynamic management on agricultural ecosystems by quantifying fungal communities in multiple habitats using metagenomics. We go on to measure biodiversity in the crop and key chemical quality parameters in the product consumed by humans. We find that the method of management significantly affects communities in soil, on plant structures, and on the developing crop in subtle but importantly different ways in terms of number, type, and abundance of species. However, management approach has no effect on communities in the final harvested juice, nor on product traits aligned with quality. This shows that while management approach impacts different habitats in the environment in different ways, this does not automatically flow onto the harvested crop.

1. Introduction

How biodiversity, and the ecosystem services it provides, responds to the way we manage natural and agricultural ecosystems is a key area of modern ecology; it impacts both conservation efforts and the cultivation of crop species which provide essential food resources (Tanentzap et al., 2015). It is commonly asserted that agriculture conflicts with natural environments, and sustainable approaches to agriculture are now receiving greater attention (Edwards et al., 2015). While we may more readily perceive how various human-mediated ecosystem interventions impact larger plants and animals, we have a poor idea about how microbial communities respond, if they respond at all, to various management approaches. Microbial communities perform essential functions in all ecosystems and play a role in directly modulating plant health, productivity, and development (Lau and Lennon, 2012; Panke-Buisse et al., 2015; Sugiyama et al., 2013). Studies to date have reported that the structure and composition of microbial communities often vary considerably over different spatial and ecological gradients (Hanson et al., 2012; Martiny et al., 2006; Nemergut et al., 2013). While the main drivers of microbial diversity may differ between ecosystems, it is generally held that terrestrial microbial communities are mostly driven by natural selection to specific habitats present in any particular environment (which would include selection pressures imposed by agrochemicals), though the significance of stochastic (neutral) effects in defining microbial community composition should not be ignored (Morrison-Whittle and Goddard, 2015; Stegen et al., 2012, 2013).

Modern agricultural management practices do not involve just one treatment but instead comprise a range of different biological, physical, and chemical treatments applied to cultivated land to maximise the health, resilience, and productivity of crop species. There is significant and seemingly growing public concern surrounding the use of agrochemical interventions, though the science evaluating their effects at the ecosystems level is sparse (Edwards et al., 2015; Tanentzap et al., 2015). Due to concerns about environmental impacts of agrochemicals, alternative philosophies to agricultural management have emerged. These alternative approaches include “organic” and “biodynamic” styles of management that, while very similar to “conventional” practices, often differ in a few notable ways. At their core, organic and biodynamic practices are primarily shaped by their philosophical opposition to the use of agrichemical pesticides and herbicides, both of which are routinely used in conventional management (Tilman et al.,

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2002). In practice, this can often manifest as differential constraints on what specific treatment decisions can be made for any one site. Organic and biodynamic practices are constrained in what they may use by local commercial-certification bodies such as BioGro^{NZ} or Demeter^{Int}. As a part of formal certification from these agencies, companies are required to conform to approved codes of practice which either heavily restricts or forbids the use of most pesticide and fertiliser products.

The subject of alternative land management philosophies is a popular albeit controversial one, and has provoked a considerable shift in many industry practices globally. It is imperative that we objectively, quantitatively assess whether different practices differentially affect ecosystems, and the crops and products that derive from them. As huge areas of the planet have been dedicated to cultivating plant species, and realising that these are not completely isolated from surrounding natural ecosystems, any effect of different management approaches to cultivation may have significant implications for the diversity and functioning of ecosystems generally. Fungal communities form a core component of natural and agricultural ecosystems, and this where we focus here as a first step.

We currently have no clear idea whether organic/biodynamic or conventional practices translate to real variation in microbial communities, nor their effect on the products deriving from these systems. Many studies have found that specific agricultural interventions can significantly impact microbial diversity in agricultural ecosystems (Čadež et al., 2010; Gomiero et al., 2011; Hartmann et al., 2015; Martins et al., 2012, 2014; Perazzolli et al., 2014; Saison et al., 2006). However, very few studies have tested whether overall treatments culminate in detectable differences in biodiversity between different agricultural philosophies. One recent long-term study found organic farming increased richness, decreased evenness, and shifted the structure of the soil microbiota compared to conventional approaches (Hartmann et al., 2015). This is an excellent study producing an important result, but only examines soil: one, albeit important, habitat in agricultural systems. To achieve a more holistic picture of the effects of different management approaches on agricultural ecosystems requires examining multiple habitats in these ecosystems. Importantly, for the consumer, the status of the produce that is cropped also needs evaluation.

Here we take an approach that samples multiple habitats in vineyard ecosystems, including the harvested juice and wine, and use DNA sequencing to enumerate the fungal communities in multiple habitats from six conventional as well as six “biodynamically” managed vineyards. We test the null hypothesis that there is no difference in the effect of management approach on microbial biodiversity across this agricultural ecosystem. We do this by breaking down and analysing different components of microbial diversity in different habitats. Since fungi are the key component that drives the fermentation of juice to wine, and produce many key quality flavour and aroma compounds as they do so, we also go onto analyse fungal diversity in juice and key fungal-derived quality flavour compounds in the wines: varietal thiols (Anfang et al., 2009; Harsch and Gardner, 2013; Masneuf-Pomarède et al., 2006; Santiago and Gardner, 2015). By quantifying community structure across multiple vineyard habitats, and key microbe-derived compounds in wine, we can more powerfully assess the ecosystem level effects of management approach that would not be possible by characterising one habitat or aspect of the ecosystem in isolation.

2. Methods

2.1. Sampling viticulture ecosystems

Soil, bark, and ripe fruit habitats were sampled from 12 commercial sauvignon blanc vineyards managed by nine different companies across the Wairau valley in the Marlborough region on the South Island of New Zealand, approx. 41°S, 173°E. The experimental design was such that $n = 6$ for each habitat for each management type; thus, 36 samples were collected from vineyards comprising six biodynamic and six conventionally managed vineyards for a fully-balanced design. All biodynamically managed vineyards had achieved BioGro™ organic certification. Approximately two weeks before harvest, around 30 g of each habitat was aseptically collected. Each sample comprised three pooled sub-samples taken across each vineyard. All samples were taken at least 5 m into the vineyard to avoid edge effects. Soil samples were taken 50 cm away from a grapevine trunk at a depth of ~10 cm. Bark samples were taken from at least 30 cm above the soil, and whole bunches of fruit were cut into sterile bags. All samples were taken with sterile tools and placed into sterile containers, and transported on ice to the laboratory for processing. Microbes were washed off fruit samples by immersion in sterile water with rocking for 30 min. The resulting solution was then centrifuged at 3000 rpm, and the resulting pellet re-suspended in 500 µl of sterile water. Soil and bark samples were homogenised mechanically using aseptic technique to increase surface area for DNA extraction.

We also collected commercially harvested juice from these same vineyards. Approximately 10 L of juice was transferred into sterile jerry cans at each winery and transported to the laboratory on ice. 50 ml of homogenised juice was centrifuged at 3000 rpm and the resulting pellets suspended in 500 µl of sterile water. Twelve juice samples directly deriving from the six biodynamic vineyards and six conventionally managed vineyards were collected. Thus, in total 48 samples were collected from the twelve vineyards and the juice derived from them.

2.2. Extraction and sequencing

All samples were frozen at −20 °C prior to processing. DNA was extracted using the Zymo Research Soil Microbe DNA MiniPrep™ kits. We empirically determined this kit was sufficient to extract DNA from all substrates. Fungal communities were characterised and enumerated by 454-sequencing of the D1/D2 region of 26S ribosomal RNA, and amplified using NL1 and NL4 primers described in Kurtzman and Robnett (2003) with unique multiplex identifiers added as appropriate. Sequencing this locus provides an effective method for taxonomic identification down to at least genus level as well as the quantification of the relative richness and abundances of fungal communities (Morrison-Whittle and Goddard, 2015; Taylor et al., 2014). All PCR products were cleaned using AmpureXP beads and their quality checked by Agilent DNA1000 chips. Juice samples were uni-directionally sequenced on a 454-junior instrument by New Zealand Genomics Limited. Vineyard communities were sequenced on a full plate of a 454 Life Sciences GS FLX instrument by Macrogen (Korea).

2.3. Sequencing pipeline

Sequence processing was carried out using Mothur v.1.30 (Schloss et al., 2009). Primers and sequences < 200 bp were removed. Low quality reads were removed using the pyronoise algorithm. Chimeric sequences produced during PCR were identified and removed using the uchime algorithm. Once the remaining high-quality sequences were bioinformatically assigned labels based on their multiplex identifier sequence, they were merged and analysed together. Unique sequences were compared to a reference database of fungal sequences. Sequences that were not identified as fungal were removed (11,105, 7.26% of all reads). The remaining 141, 940 fungal sequences were then aligned using a fungal reference database and clustered at > 98% identity.

The 98% identity threshold was used to approximate clusters of fungal species (Kurtzman and Robnett, 2003; Romanelli et al., 2010) and was the lowest level of molecular operational taxonomic unit (MOTU) in this study. Any MOTU that was represented by a single read (a singleton) was conservatively removed from the sequence pool. To effect equal sampling effort for these DNA sequences, reads were sub-sampled (rarefied) to the sample with the lowest read count, resulting in 509 reads per sample. Representative sequences of each MOTU were