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Species richness influences wine ecosystem function through a dominant species

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ABSTRACT

Increased species richness does not always cause increased ecosystem function. Instead, richness can influence individual species with positive or negative ecosystem effects. We investigated richness and function in fermenting wine, and found that richness indirectly affects ecosystem function by altering the ecological dominance of *Saccharomyces cerevisiae*. While *S. cerevisiae* generally dominates fermentations, it cannot dominate extremely species-rich communities, probably because antagonistic species prevent it from growing. It is also diluted from species-poor communities, allowing yeasts with lower functional impacts to dominate. We further investigated the impacts of *S. cerevisiae* and its competitors in high- and low-functioning wine communities, focusing on glucose consumption as an ecosystem function. *S. cerevisiae* is a keystone species because its presence converts low-functioning communities to communities with the same function as *S. cerevisiae* monocultures. Thus, even within the same ecosystem, species richness has both positive and negative effects on function.

1. Introduction

The number of species in a community (species richness) directly and indirectly influences productivity, consumption, decomposition, and other community functions (Hooper et al., 2005; Nielsen et al., 2011). Direct effects of richness on function are well studied, especially in plant ecosystems (Tilman et al., 1996; Hector et al., 1999; Reich et al., 2012). However, we know less about indirect richness effects, which may be particularly important in microbial communities (Nielsen et al., 2011). Specifically, we do not fully understand how the influence of species richness on individual species changes community function.

Richness may indirectly impact community function through dominant and keystone species. Dominant species are species represented by a relatively large number of individuals in a community (Hillebrand et al., 2008). Keystone species are frequently defined to be species with disproportionately high functional impacts with respect to representation; we use this definition, although there are competing definitions in the literature (Mills et al., 1993; Power et al., 1996). A keystone species may also become dominant over time after being introduced to a community in small numbers. Richness and function can correlate positively when few species contribute to community functioning, or negatively when few species inhibit function, because species-rich communities are more likely than species-poor communities to contain dominant or keystone species (Duffy et al., 2003; Dangles and Malmqvist, 2004; Jiang et al., 2008; Tolkkinen et al., 2013). For example, functionally impactful keystone yeast strains use resources wastefully and decrease overall community function (Pfeiffer et al., 2001; MacLean and Gudelj, 2006). However, species-rich communities may also be more likely to include competitors or facilitators that modify the performance of dominant and keystone species (Toljander et al., 2006), and some keystone species may indirectly influence function by decreasing community species richness over time as they become dominant (Gaertner et al., 2009; Hejda et al., 2009).

Richness can also directly influence community function. Complementary resource use among species (niche complementarity) explains positive correlations between richness and function in most plant and some heterotrophic communities (Loreau and Hector, 2001; Setälä and McLean, 2004; Reich et al., 2012;

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Zuppinger-Dingley et al., 2014). Facilitation among species can also lead to positive richness-function correlations (Tiunov and Scheu, 2005). For example, diverse suspension feeding communities slow water flow rates and capture more particles than single-species communities (Cardinale et al., 2002). In contrast, inter-species competition often leads to negative richness-function correlations. Direct antagonistic interactions (*e.g.,* toxin production) are most frequently invoked to explain negative correlations (Fukami et al., 2010; Jousset et al., 2011; Becker et al., 2012). Communities can have hump-shaped richness-function curves when competitive interactions shape ecosystem function at high richness, while niche complementarity or facilitation shapes ecosystem function at low richness (Toljander et al., 2006; Costantini and Rossi, 2010).

We investigated interactions among species richness, dominant species, and community function in uninoculated grape must, the precursor to wine. Must is a mixture of crushed grapes and resident microbes, including microbes introduced from grape surfaces, winery equipment, and by vectors including insects and wine-makers (Fleet and Heard, 1993; Mortimer and Polsinelli, 1999; Stefanini et al., 2012; Bokulich et al., 2013). Its fungal community contains the well-studied dominant yeast species *Saccharomyces cerevisiae*. *S. cerevisiae* usually dominates must over successional time: it is generally present in low frequencies in young must, and its increased frequency with time correlates with decreased species richness (Cocolin et al., 2000; Torija et al., 2001; Nisiotou et al., 2007). As with other fermented foods, must is an experimentally tractable partially natural system (Wolfe and Dutton, 2015). It is more easily manipulated than many natural systems, including soil, because many must fungi are culturable and species richness is relatively low. It is also more relevant to natural systems than many artificially assembled laboratory communities, which may contain community members that have not previously encountered one another (Hom and Murray, 2014).

We used observations and experiments to understand the impact of species richness on *S. cerevisiae* dominance in must, and further, the impacts of species richness and *S. cerevisiae* on must community function. We first confirmed that *S. cerevisiae* is a dominant species in must fermentations by tracking the fungal community compositions of several fermentation vats using high-throughput sequencing. We compared *S. cerevisiae* frequency with species richness (the number of species present) and evenness (the uniformity of species' relative frequencies (Pielou, 1977)) over successional time.

We then looked for correlations between fungal species richness and community function in microcosms made from young winery must. We focused on two community functions related to primary consumption: glucose consumption and biomass production. We chose these functions because they measure two different aspects of primary consumption: uptake of one common nutrient and overall conversion of nutrients to biomass. However, they are not the only functions performed by the must microbial community: microbes also consume fructose, other sugars, and other nutrients, and they engage in secondary metabolism, including production of aromatic flavour compounds (Fleet, 1993). Microcosm species richness was altered with serial dilutions: dilution removes rare taxa from a community while retaining common taxa. After incubation, microcosms were assayed for the two ecosystem functions and species composition. Our dilution treatments most likely had similar effects on bacterial richness as on fungal richness, but we focused on fungal richness because we were specifically interested in the guild containing *S. cerevisiae* and organisms with similar effects on the ecosystem. We hypothesized that *S. cerevisiae* presence drives the relationship between species richness and ecosystem function because species-rich microcosms are more likely to contain *S. cerevisiae* than species-poor microcosms.

In addition to being numerically dominant, *S. cerevisiae* may be a keystone species in must. We investigated the influences of several individual yeast isolates, including *S. cerevisiae*, on ecosystem function by introducing them to communities derived from high-functioning and low-functioning microcosms. We compared impacts of each tested yeast on artificial community function, and we expected *S. cerevisiae* to have disproportionately higher functional impacts than other yeasts if it is a keystone species.

2. Methods

2.1. Must collection

All must samples were collected in October and November 2013 from the San Polino winery in Montalcino, Italy. The winery has been operated by its current owners since 1994, who have exclusively practiced uninoculated fermentation since 2003. Ten winery fermentation vats are filled yearly with must from Sangiovese grapes harvested from five vineyards, all within 5 km of the winery. Filled vats are closed to the outside environment. Limited dispersal is possible among vats because the winemakers use the same equipment to fill, mix, and transfer must among vats. Equipment is cleaned, but not sterilized, between usages. Vat volumes range from 3000 to 3800 L, and fermenting must remains in the vats for about a month before it is filtered and aged in oak barrels for years. Mature wine is then blended, bottled, and eventually enjoyed as fine Brunello di Montalcino, Rosso di Montalcino, and Sant Antimo wines.

We collected must samples from five vats approximately every 12–24 h over 13 d starting from the day the first vat was completely filled. One ml of grape must was collected at each timepoint. To prevent further fermentation during storage and transport, we centrifuged must samples for 5 min at 6000 rpm in a tabletop microcentrifuge and fixed the pelleted cells in 250–500 μl 100% ethanol. Samples were stored at ambient temperature until DNA extraction (19 d or less), and alcohol was removed from each sample before DNA extraction. DNA was extracted using the MasterPure™ Yeast DNA Purification Kit (Epicentre, Madison, Wisconsin, USA) following the manufacturer's instructions.

Must samples were also collected from six vats or vat mixtures once fermentation was completed, after the winemakers had filtered the fermented must. Post-filtration samples were transported at ambient temperature without treatment for 7 d before DNA extraction. We did not expect further fermentation in post-filtration samples because alcohol concentration was more than 14% in each vat. The winemakers combined the contents of some vats during filtration, and two post-filtration samples were mixtures of two vats each. When comparing diversity among vats, we assigned each of these two mixtures to the vat which contributed the most volume to the mixture (*i.e.,* a sample consisting of 54% Vat 17 must and 46% Vat 1 must was analysed as Vat 17 and a sample consisting of 67% Vat 22 must and 33% Vat 20 must was analysed as Vat 22). The total number of must samples collected ranged from 6 to 23 per vat. Two additional vats were only sampled once, after filtration.

2.2. Microcosm experiment

We tested the relationship between species richness and ecosystem function in small volumes of fermenting grape must (microcosms). We prepared ten replicates each of five dilution treatments plus uninoculated controls (Fig. S1). Treatments included undiluted unsterilized grape must and unsterilized must serially diluted 1:10, $1:10^3$, $1:10^5$, and $1:10^7$ with 0.22 μm-filter-