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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-

sterilized must (D0, D1, D3, D5, and D7, respectively). One millilitre was removed from each inoculated microcosm for DNA sequencing before incubation, and the remaining 10 ml microcosms were incubated for 14 d at 30 °C with 200 rpm shaking. Inoculum sizes ranged from about 50 to 5×10^8 colony-forming units (CFUs) per 10 ml microcosm. All must originated from a single vat (Vat 17). Must was collected 64 h after the vat was filled, and transported on ice for 24 h before microcosm preparation.

In addition to the cells harvested before incubation, we also harvested cells for DNA sequencing and measured microcosm biomasses and glucose concentrations after 14 d. Cells were harvested from all inoculated microcosms by centrifuging 1 ml of each microcosm (10 min at 16,837 rcf) and removing the supernatant. DNA was extracted from each pellet as described above. To measure biomass, we centrifuged a second 1 ml from each microcosm, dried each pellet at 80 °C for 38 h, and weighed pellets on a microbalance. Supernatants were retained for glucose concentration assays. We decolourized supernatants by incubating 250 μl of filter-sterilized supernatant with 25–50 mg activated carbon pellets for 24 h. Glucose concentration was then measured using a Glucose (HK) Assay Kit (Sigma®, St. Louis, Missouri, USA), according to the manufacturer's instructions. Microcosm glucose values less than 0.14 mg ml^{-1} were assumed to be below the limit of kit detection, and were assigned a value of zero. We preserved microcosms for follow-up culturing after 16 d by mixing 0.5 ml microcosm aliquots with 0.5 ml 40% glycerol and storing the aliquots at −80 °C.

2.3. MiSeq® amplicon sequencing

Fungal ITS2 amplicons of 65 vat samples, 100 microcosm samples, and four constructed control samples were sequenced using MiSeq® (Illumina®, San Diego, California, USA). Constructed control samples were known numbers of CFUs of three grape must yeasts (*S. cerevisiae*, *Hanseniaspora uvarum*, and *Metschnikowia* sp.) in grape must. We processed and sequenced constructed control communities alongside vat and microcosm communities in order to better understand biases and errors in our sampling and sequencing protocols. DNA was extracted from constructed control samples as described above for microcosm samples.

LGC Genomics (Berlin, Germany) prepared and sequenced a barcoded amplicon library consisting of all 169 samples amplified using the fungal-specific primer pair fITS7/ITS4 (White et al., 1990; Ihrmark et al., 2012). Technicians at LGC Genomics diluted each DNA extract 1:50, and amplified samples using barcoded primers. Both forward and reverse barcodes were unique for each sample. PCR reactions consisted of 1 μl dilute template, 15 pmol each barcoded primer, 1.5 units MyTaq™ DNA Polymerase (Bioline, London, UK), and 2 μl BioStab PCR Optimizer II (Sigma-Aldrich, St. Louis, Missouri, USA) in 20 μl MyTaq buffer. Reactions were cycled for 2 min at 96 °C, then for 40 cycles of 96 °C for 15 s, 50 °C for 30 s, and 70 °C for 60 s. Amplicon concentration was then determined using gel electrophoresis, and about 20 ng of each amplicon was pooled into 48-sample amplicon pools. Amplicon pools were purified using both AMPure® XP beads (Beckman-Coulter, Krefeld, Germany) and MinElute® columns (Qiagen, Hilden, Germany) to remove primer dimers. LGC then constructed Illumina libraries using the Ovation® Rapid DR Multiplex System (Qiagen, Hilden, Germany), and ran samples on Illumina MiSeq® cartridges using V2 or V3 chemistry.

Sequencing produced a total of 8,098,202 paired-end contigs. LGC genomics sorted FASTQ files by barcode, removed adapter and barcode sequences, and discarded sequences with missing or incompatible barcodes using bcl2fastq version 1.8.4 (Illumina, San Diego, California, USA) and in-house scripts. We then used Mothur version 1.33.3 to join paired ends into contigs (Schloss et al., 2009). Mothur also removed 757,652 sequences with ambiguous bases, homopolymers longer than 18 bp, or length not between 250 and 550 bases. The remaining sequence dataset was composed of 1,580,442 unique sequences. Of these, we removed all instances of the 19,617 unique sequences (22,404 total removed sequences) that were predicted to be chimeric using the *de novo* UCHIME interface in Mothur (Edgar et al., 2011), leaving 1,560,825 unique and 7,318,146 total sequences. We clustered operational taxonomic units (OTUs) at 98.5% similarity using the BLAST-based reference method in QIIME (Altschul et al., 1997; Caporaso et al., 2010). OTUs were clustered against the dynamic UNITE database version 6, release date September 10, 2014, containing 21,185 total reference and representative sequences (Kõljag et al., 2013). OTUs were assigned the same taxonomic identity as the UNITE sequence to which they were clustered. Sequences below 98.5% similarity to a UNITE sequence were discarded (1,285,298 sequences). OTUs represented only once in our dataset (singleton OTUs) were assumed to be sequencing errors, and were removed (87 sequences). The final dataset was composed of 6,032,761 sequences clustered into 524 OTUs. The operational taxonomic unit (OTU) table including taxonomy assignments to species and metadata are included in Tables 1, 2, 4, and 5 in the data paper linked to this article (Boynton and Greig, 2016). FASTQ files were deposited into the GenBank Sequence Read Archive (accession SRP073276). We also produced a yeast-only dataset composed of 2,005,021 sequences clustered into 84 yeast OTUs, which included only genera listed in the table of contents of *The Yeasts, a Taxonomic Study* (Kurtzman et al., 2011), a reference for ascomycete and basidiomycete yeast taxonomy.

We processed the sequencing-derived OTU table before comparing diversity among microcosm and vat samples. First, we divided the OTU table into microcosm and vat datasets. We then subsampled the microcosm and vat datasets to 5008 and 7548 sequences per sample, respectively, to correct for differences in sampling depth among samples. We discarded all samples with fewer sequences (2 microcosm and 6 vat samples, Fig. S2). We chose 5008 and 7548 sequences because sampling rarefaction curves began to asymptote at about these values, and the values were low enough to minimize the number of discarded samples. We also discarded one outlier microcosm sample which had OTU richness greater than three standard deviations above the mean richness for its treatment and timepoint.

2.4. Sequencing and experimental reliability

We analysed four constructed control communities to understand errors in our experimental protocol. Errors can come from a variety of sources, including DNA contamination in samples and biases in ITS copy number, DNA extraction, PCR, sequencing, OTU clustering, and taxonomy assignment. Of the three species inoculated into constructed control communities, *S. cerevisiae* sequences were detected more frequently than expected, and *H. uvarum* and *Metschnikowia* sp. sequences were detected less frequently than expected (Fig. S3). These biases may be due to differences in ITS copy number, sequencing efficiency, amplicon length, or primer annealing efficiency among taxa (Maleszka and Clark-Walker, 1993; Bokulich and Mills, 2013). Sequencing overestimated OTU richness in constructed control communities (10–29 OTUs in non-rarefied constructed control communities compared to three inoculated taxa). Richness overestimates may be due to DNA contamination from grape must, sequencing errors, or errors in OTU clustering. We tested several other OTU clustering protocols on a randomly chosen subset of 100,000 sequences; all tested protocols overestimated species richness more than the BLAST-based protocol used (Table S1). When analysing vat and microcosm datasets, we assumed biases in species richness estimates were consistent across samples.

Constructed control community taxonomy assignment was reliable to genus but not species level. We report vat and microcosm taxon assignments to genus, except for *Saccharomyces*. To confirm *Saccharomyces* sequence species assignment, we re-clustered sequences assigned to the genus *Saccharomyces* against a custom reference database of five *Saccharomyces* species (*S. cerevisiae*, *Saccharomyces paradoxus*, *Saccharomyces kudriavzevii*, *Saccharomyces mikatae*, and *Saccharomyces uvarum*) at 99.2% sequence similarity. Reference sequences originated from the Saccharomyces Genome Resequencing Project or sequenced *Saccharomyces* genomes deposited in GenBank (Cliften et al., 2003; Kellis et al., 2003; Liti et al., 2009). We chose reference *Saccharomyces* species to include the wine yeast *S. cerevisiae*, its three closest relatives, and one of its two most distant relatives in the *Saccharomyces sensu stricto* clade (Almeida et al., 2014; Boynton and Greig, 2014). 99.95% of control, vat, and microcosm sequences were assigned to *S. cerevisiae*. We assumed that the remaining 0.05% of sequences were erroneously assigned, and that all *Saccharomyces* sequences in the dataset are *S. cerevisiae*. We did not collapse all *Saccharomyces* sequences into a single OTU because it was infeasible to reassign all assigned OTUs in the dataset, and we did not want to bias species richness estimates.

2.5. Keystone species assay

To determine whether individual taxa are keystone species, we measured glucose consumption in artificial communities composed of the microbes of different D7 microcosms, supplemented with additional experimental species. We first isolated yeast clones from each D7 microcosm after incubation. Frozen microcosm material was diluted and plated on solid YPD media (1% yeast extract, 2% peptone, 2% dextrose, 2.5% agar). Twenty colonies were randomly selected from each replicate microcosm. The ITS region of each colony was sequenced using the primers ITS1/ITS4 to identify isolates to genus or species using the NCBI BLAST database (White et al., 1990; Altschul et al., 1997). Several strains were not identifiable using the ITS1/ITS4 primer pair, and we further sequenced these strains using the primer pairs 5.8S/LR3, EF1-983F/EF1-2212R, and/or fRPB2-5F/RPB2-7R (Vilgalys and Hester, 1990; Rehner and Buckley, 2005; Schoch et al., 2012). Sequences were deposited into GenBank (accession numbers KX078411-KX078449).

We then created artificially assembled communities by mixing individual yeast isolates with inocula sampled from each of four D7 microcosms at the end of the microcosm experiment. Culturing produced yeasts from four different genera (Table 1), and we included five isolates from each genus as experimental replicates within a genus. Five was the maximum possible replicate number because we only isolated five *Cryptococcus* clones. We combined yeasts with inocula derived from each of four microcosms, chosen at random to represent every cultured community composition (microcosm replicates 1, 2, 4, and 8). No genus was combined with inoculum from a microcosm that contained culturable representatives from that genus: for example, no *Saccharomyces* isolates were combined with microcosm replicate 1, because we found *Saccharomyces* when culturing from this microcosm. Inocula were prepared, and artificial communities were grown, in filter-sterilized commercial grape juice (Aldi-Nord, Essen, Germany). To produce inocula, each yeast isolate and 30 μl of each frozen microcosm stock was individually grown in grape juice overnight at room temperature. We determined yeast and microcosm inoculum sizes by diluting inocula and counting CFUs on YPD media.

Table 1
Counts and identities of cultured yeast isolates from D7 microcosms.

Microcosm replicate	Glucose consumed (%)	Taxa	# Isolates
1	100	*Saccharomyces cerevisiae*	20
2	35	*Hanseniaspora uvarum*	14
		Nakazawaea ishiwadae	6
3	32	*S. cerevisiae*	20
4	38	*H. uvarum*	15
		Cryptococcus wieringae	3
		Cryptococcus tephrensis	1
		Cryptococcus carnescens	1
5	32	*H. uvarum*	20
6	42	*H. uvarum*	20
7	100	*S. cerevisiae*	20
8	38	*H. uvarum*	20
9	34	*H. uvarum*	20
10	100	*S. cerevisiae*	20

To produce artificial communities, small amounts of each yeast inoculum were mixed with larger amounts of each microcosm inoculum; we aimed to inoculate each artificial community with 10% yeast CFUs and 90% microcosm CFUs, although there was considerable variation in relative inoculum sizes (mean = 11% yeast CFUs, standard deviation = 13%). A total of 50 artificial communities were produced (including four yeast genera, five replicate isolates per yeast, and four microcosm inocula. The experimental design was not fully factorial). We also produced control artificial communities composed of uninoculated juice, each yeast alone (four yeast genera x five replicate isolates), and each microcosm inoculum alone (four microcosm inocula x five identical replicates). Artificial communities were grown at 30 °C for 7 d with 200 rpm shaking. Final glucose concentration was assayed as described above. We compared glucose consumption of each yeast alone, each microcosm inoculum alone, and artificial communities composed of one yeast and one microcosm inoculum.

2.6. Statistical analyses

OTU richness, Pielou's evenness (Pielou, 1977), and percentage of sequences assigned to *Saccharomyces* were calculated for each vat and microcosm sample. We modelled diversity indices over time in winery vats using linear regression. To normalize data, we transformed time by $\log_{10}(x+1)$, and percentage *Saccharomyces* sequences by $\log_{10}$. Richness and evenness were left untransformed. We also compared OTU richness and evenness among microcosms at the beginning and end of the experiment (0 and 14 d) using a full mixed effects ANOVA with timepoint nested in treatment, nested in replicate.

Glucose and biomass were compared among microcosm diversity treatments. We transformed microcosm glucose into percentage total glucose consumed by normalizing glucose concentrations to uninoculated controls. Glucose measurements below the kit minimum detection threshold were assumed to contain no glucose (*i.e.*, the community consumed 100% of available glucose). We also corrected biomass values by subtracting average uninoculated control values from each treated biomass value. Biomasses were compared among treatments using one-way ANOVA. We tested for correlations among biomass, glucose consumption, and percentage of sequences assigned to *S. cerevisiae* using Kendall's rank correlation tau.

We developed a keystone index for the yeast component of each artificial community to describe disproportionate influences of a yeast on ecosystem function. A yeast was considered a keystone species in an artificial community if community glucose consumption was more similar to the yeast monoculture than expected based on the yeast and microcosm community components. In any community, the yeast's keystone index (*KI*) ranges from −1 to 1, and centres on expected glucose consumption, the weighted average of

each yeast or microcosm component when grown alone. $KI = 1$ when an artificial community consumes the same amount of glucose as the yeast component alone, −1 when it consumes the same amount of glucose as the microcosm component alone, and 0 when it consumes the expected amount of glucose. Expected glucose (*exp*) is the average glucose consumption of an artificial community's yeast and microcosm components when grown alone, weighted to inoculum size:

$$exp = yp_y + m\left(1 - p_y\right)$$

Where y is the amount of glucose consumed by the yeast component alone, m is the amount of glucose consumed by the microcosm component alone (mean of five replicates), and p_y is the proportion of yeast CFUs in the inoculum.

When glucose consumed (*obs*) is between *exp* and *y*:

$$KI = (obs - exp)/(y - exp)$$

When *obs* is between *exp* and *m*:

$$KI = (obs - exp)/(exp - m)$$

When *obs* does not lie between *m* and *y*, $KI = -1$ if *obs* is closer to *m*, and 1 if *obs* is closer to *y*.

We compared keystone indices between yeasts grown with the same microcosm inoculum using multiple Wilcoxon rank sum tests and a Holm-Bonferroni p-value adjustment (Holm, 1979). We also compared each community component (yeasts and microcosm inocula) when grown alone against 0% glucose consumed using one-sample t-tests and a Holm-Bonferroni p-value adjustment. In some cases, all replicates in a treatment did not leave detectable glucose and could not be compared using a t-test; we assumed these treatments consumed 100% of available glucose.

All statistical analyses were conducted using R version 3.1.1 and the vegan, GUniFrac, nlme, and multcomp packages (Horthorn et al., 2008; Chen, 2012; Oksanen et al., 2014; Pinheiro et al., 2014; R Core Team, 2014).

3. Results

3.1. Vat diversity

OTU richness, an estimate of the number of species present in the vats, decreased in vats over time ($F_{1,57} = 22.74$, $p < 0.001$, adjusted $R^2 = 0.27$; Fig. 1). OTU evenness, an estimate of the uniformity of relative species abundances in the vats, did not change significantly over time ($F_{1,57} = 0.72$, $p = 0.40$, Fig. S4A), but *S. cerevisiae* frequency increased significantly over time ($F_{1,57} = 46.7$, $p < 0.001$, adjusted $R^2 = 0.44$, Fig. S4B).

S. cerevisiae is the dominant species late, but not early, in grape-must succession. Plant-associated fungi, especially *Aureobasidium pullulans* dominated young grape must communities (Figs. S5, S6, OTU table provided in (Boynton and Greig, 2016), Table 5), and *S. cerevisiae*, as well as other yeasts in the Saccharomycetales, incompletely replaced plant-associated fungi after about 5–7 d. By the end of fermentation, *S. cerevisiae* sequences dominated most vats, although sequences from plant-associated fungi persisted throughout fermentation. Plant-associated OTUs included members of the orders Dothideales, Capnodiales, and Helotiales. Yeast OTUs included basidiomycete and ascomycete yeasts, especially members of the genera *Saccharomyces*, *Cryptococcus*, and *Debaryomyces*.

3.2. Microcosm diversity

Diluting unsterilized grape must with filter-sterilized must in the microcosms reduced initial OTU richness and increased initial OTU evenness (Fig. 2). Both diversity indices levelled off between treatments D3 and D7. Richness and evenness patterns were consistent with an undiluted fungal community containing few common and many rare species, because dilution removes rare species from microcosms. Microcosm species compositions qualitatively resembled vat species compositions (Fig. S7).

After 14 d of incubation, microcosm OTU richness decreased in the two less dilute and initially more diverse treatments (D0 and D1), but did not change in the three more dilute and initially less diverse treatments (D3, D5, and D7; mean = 35 OTUs for all microcosms after 14 d, Fig. 2A). Interestingly, OTU evenness increased after 14 d in undiluted microcosms (D0), but decreased or stayed the same in diluted microcosms (Fig. 2B). The final microcosm OTU evennesses was driven by *S. cerevisiae* dominance in intermediate dilutions: the final frequencies of *S. cerevisiae* sequences were highest in the intermediate dilutions D1-D5, and lowest in the undilute D0 and most dilute D7 treatments, although there was considerable variation within treatments (Fig. 3).

3.3. Microcosm ecosystem function

Microcosm glucose consumption showed a hump-shaped pattern with respect to dilution treatment (Fig. 4, glucose data provided in (Boynton and Greig, 2016), Table 3). All microcosms in treatments D1-D5 consumed all available glucose (100% of 119.4 mg ml^{-1} glucose in grape must), while D0 microcosms did not consume glucose completely (mean = 77%). Glucose consumption was bimodal in D7 microcosms: three of ten replicate microcosms consumed all available glucose, and the remaining seven replicates consumed a mean of only 36% of the available glucose.

Microcosm glucose consumption correlated with ending *S. cerevisiae* sequence frequency (frequency after 14 d) across all microcosm treatments (Kendall's $\tau = 0.55$, $z = 4.83$, $p < 0.001$, Fig. 3); the correlation was most dramatic within treatment D7. Within D7, the three replicates that completely consumed glucose had more than 55% *S. cerevisiae* sequences after incubation, while the seven replicates that did not consume glucose completely had less than 25% *S. cerevisiae* sequences after incubation. Replicate microcosms with different glucose consumptions differed even more dramatically in the composition of culturable isolates. All 20 clones from the three microcosms with complete glucose consumption were *S. cerevisiae* (Table 1). The other seven microcosms contained *H. uvarum, Nakazawaea ishiwadae*, *Cryptococcus wieringae, Cryptococcus tephrensis,* and/or *Cryptococcus carnescens*.

Microcosm biomass decreased with decreasing diversity treatment, although the difference was only statistically significant between intermediate treatments and D7 ($F_{3,36} = 10.78$, $p < 0.001$, Fig. S8A, biomass data provided in (Boynton and Greig, 2016), Table 3). Microcosms from treatment D7 produced an average of 25 mg less biomass per 10 ml microcosm than microcosms from treatments D1-D5. We do not report D0 microcosm biomasses because D0 microcosms contained undilute grape solids. Biomass correlated with percentage *S. cerevisiae* sequences across all measured treatments (Kendall's $\tau = 0.25$, $z = 2.2$, $p = 0.03$, Fig S8B).

3.4. Keystone species assay

When grown alone in grape juice, glucose consumptions of individual yeast and microcosm inocula ranged from not significantly different from 0% (*Cryptococcus*, *Hanseniaspora*, microcosm 4, adjusted $p = 0.47$, 0.09, and 0.65, respectively) to 100% of available

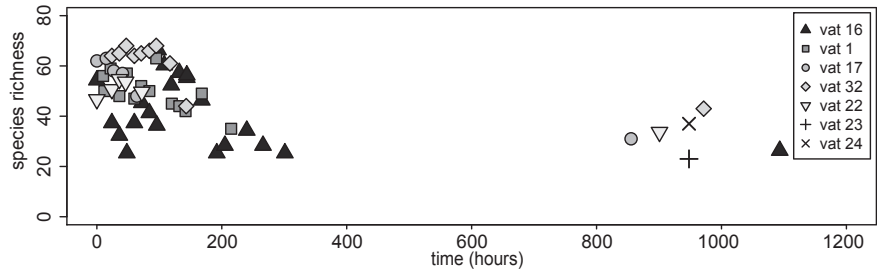

Fig. 1. Grape must species richness in five winery vats over time.

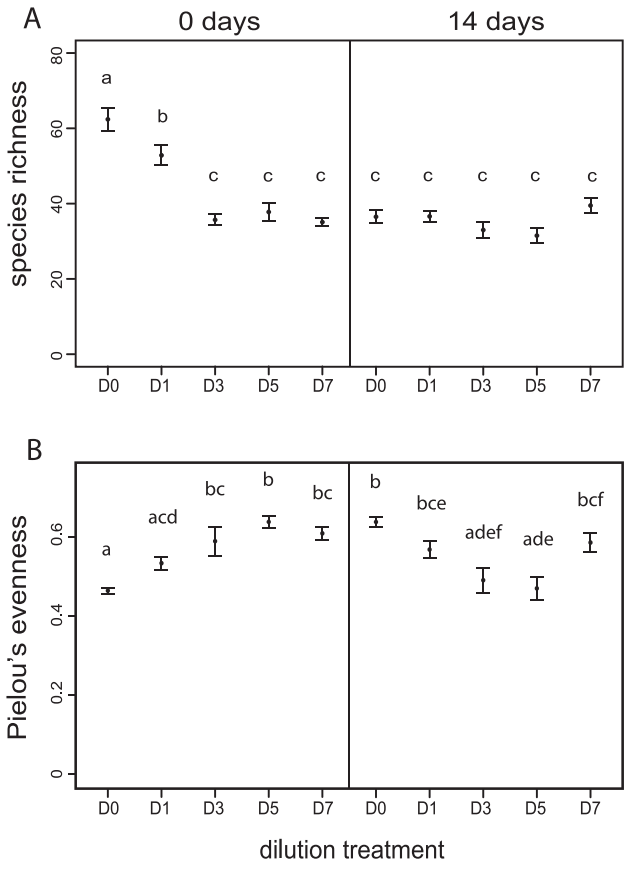

Fig. 2. Species richness (A) and evenness (B) in microcosms at two timepoints. Points are means and bars are standard errors. Different letters represent significantly different values (adjusted $p < 0.05$, Tukey post-hoc contrasts).

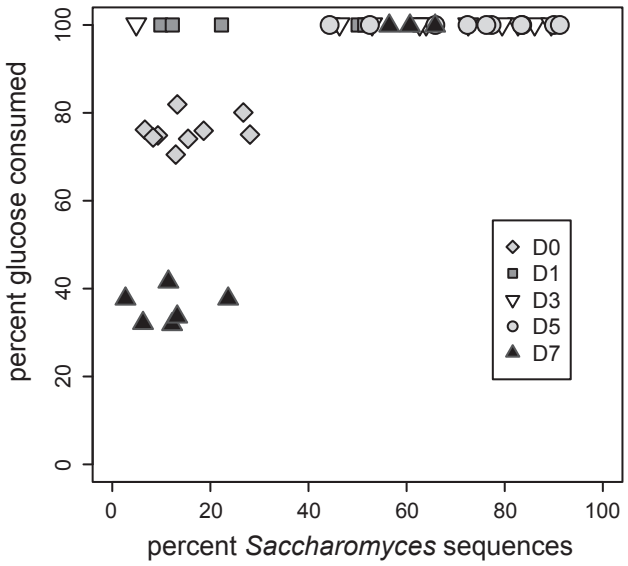

Fig. 3. Correlation between final percent sequences assigned to *Saccharomyces* and percent glucose consumed (Kendall's $\tau = 0.55$, $z = 4.83$, $p < 0.001$).

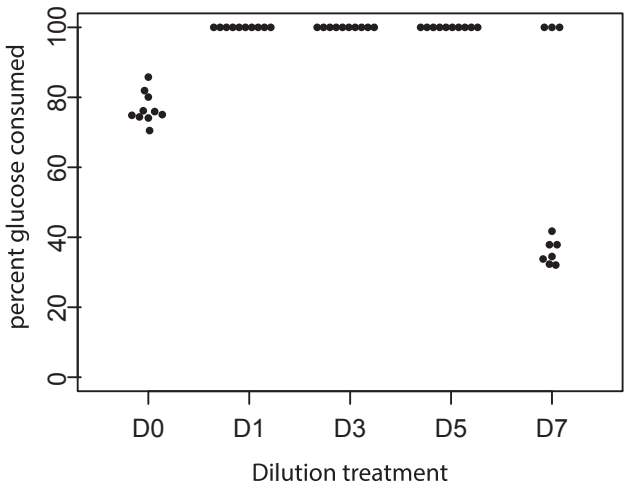

Fig. 4. Microcosm glucose consumption in diversity treatments. Each dilution treatment has ten measurements.

glucose (*Saccharomyces*, *Nakazawaea*, microcosm 1; Fig. 5A, B). While microcosm inocula followed the same trend as must microcosms when regrown in grape juice, there were slight differences in glucose consumption (Table 1; Figs. 4 and 5B), which we attribute to the different compositions of winery must and commercial grape juice. Glucose concentrations of uninoculated must and juice were 119.4 and 72.7 mg ml^{-1}, respectively.

Saccharomyces, *Nakazawaea*, and *Cryptococcus* isolates had positive keystone indices when present in some or all artificial communities, and *Saccharomyces* keystone indices were consistently larger than those of other yeasts (Fig. 5C). When culturable *S. cerevisiae* was present in an artificial assemblage, either as the yeast component of an artificial community or as part of microcosm 1 inoculum, 100% of available glucose was always consumed (Fig. 5, S9). Artificial assemblages without *S. cerevisiae* did not completely consume all available glucose. Expected and observed glucose concentrations, inoculum sizes, and keystone indices are provided in (Boynton and Greig, 2016), Tables 6 and 7.

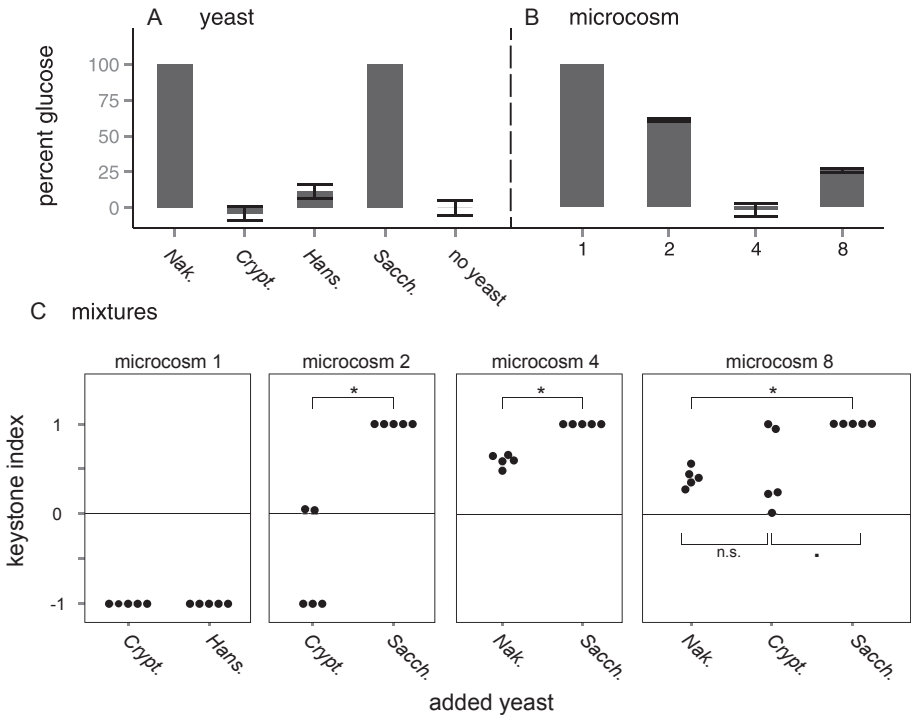

Fig. 5. Glucose consumption in artificially assembled communities. (A) glucose consumed by each yeast when grown alone. (B) glucose consumed by inoculum made from each microcosm alone. (C) keystone indices for each artificial community (n = 5). It was not possible to calculate a keystone index for *Nakazawaea* in microcosm 1 because both *Nakazawaea* and microcosm 1 consumed 100% of available glucose when grown alone. Keystone indices of *Hanseniaspora* and *Cryptococcus* in microcosm 1 were not compared against each other because all values for both yeasts were −1. "*Crypt.*" = *Cryptococcus*, "*Hans.*" = *Hanseniaspora*, "*Sacch.*" = *Saccharomyces*, and "*Nak.*" = *Nakazawaea*. Bars in (A) and (B) are means and error bars are standard errors. Asterisks, ".", and "n.s." in (C) indicate pairs of significantly, marginally significantly, and not significantly different communities, respectively (Wilcoxon's rank sum test, adjusted $p < 0.05$, $= 0.0507$, and >0.0507, respectively).

4. Discussion

4.1. *S. cerevisiae* is a dominant and keystone yeast in grape must

S. cerevisiae frequency in winery vats increased over time and correlated with decreased species richness (Fig. 1, S4). While sequence frequencies do not always reliably reflect absolute organism frequencies in a sample (Fig. S3), changes in relative sequence frequencies across samples can predict increases or decreases in organism frequencies (Amend et al., 2010). Our observations of increasing *S. cerevisiae* frequency during succession are consistent with previous culturing and sequencing-based observations of spontaneous must fermentation (Cocolin et al., 2000; Torija et al., 2001; Goddard, 2008; Bokulich et al., 2011). Vat evenness did not change with time, suggesting that species other than *S. cerevisiae* were dominant early in succession, but were replaced by *S. cerevisiae*. We did not measure biomass in vats, and do not know whether increased *S. cerevisiae* frequency corresponds to larger numbers of *S. cerevisiae* cells at any point in time, or if total biomass decreased with time while *S. cerevisiae* increased in relative frequency.

S. cerevisiae's ecological dominance drives must ecosystem function. Final *S. cerevisiae* abundance correlated with glucose consumption and biomass production in microcosms (Fig. 4, S8B). In the D7 treatment, the three microcosms that consumed all available glucose (high-functioning microcosms) had high *S. cerevisiae* frequency, while the seven D7 microcosms that did not consume glucose completely (low-functioning microcosms) did not. Culturing confirmed the general pattern: all twenty isolates from high-functioning microcosms, and no isolates from low-functioning microcosms, were *S. cerevisiae*. In contrast, while biomass and *S. cerevisiae* frequency correlated weakly across treatments (Fig. S8B), biomass did not correlate with glucose consumption among D7 microcosms (Kendall's $\tau = 0.07$, $z = 0.28$, $p = 0.78$). We suspect that *S. cerevisiae* weakly drives biomass production. This weak relationship between *S. cerevisiae* and biomass may be a result of the multiple factors influencing biomass production, including cell number, cell size, and resource use efficiency. All of these factors may vary among species, strains within a species, or sets of interspecies interactions; glucose consumption may have a much simpler relationship with *S. cerevisiae* presence because it is a simpler process.

D7 microcosms had both low species richness and small inoculum sizes, and it is possible that low-functioning D7 communities did not have enough time to completely consume microcosm glucose. To investigate this possibility, we tracked the growth of single-strain cultures with different inoculum sizes in commercial grape juice. We inoculated 10 ml cultures with approximately 20 or 2×10^9 *Hanseniaspora* or *Saccharomyces* CFUs (just below D7 or just above D0 inoculum sizes), and grew them for seven days under the same conditions as the microcosms (Fig. S10). We chose *Hanseniaspora* and *Saccharomyces* because all D7 microcosms contained one of these two yeasts. All cultures reached a steady optical density at 600 nm (OD_{600}) after two days, and we assume maximum ecosystem functioning was reached at maximum cell number. However, ending OD_{600} was lower in *Hanseniaspora* cultures with small inoculum sizes than *Hanseniaspora* cultures with large inoculum sizes. All D7 cultures did indeed have enough time to consume all glucose possible, but it was not possible to consume all available glucose in cultures with only *Hanseniaspora*.

The behaviour of *Hanseniaspora* in monocultures may indicate one mechanism for low ecosystem functioning in communities

without *Saccharomyces*. While *Saccharomyces* cultures all reached similar OD_{600} regardless of inoculum size, ending *Hanseniaspora* OD_{600} depended on inoculum size. In fact, *Hanseniaspora* OD_{600} changed little from starting OD_{600} in juice cultures, regardless of inoculum size (Fig. S10). We attribute low *Hanseniaspora* growth under our experimental conditions to a low optimal growth temperature of *Hanseniaspora* (Salvadó et al., 2011). *Hanseniaspora* survives at 30 °C—the temperature at which we incubated microcosms, and a temperature frequently reached under winemaking conditions—and even divides for a short period of time before heat stress impedes further division. It cannot, however, divide for long periods of time under our experimental conditions. In contrast, *Saccharomyces* continues to divide until it reaches high OD_{600} values even when inoculated in small numbers. It is likely that *Saccharomyces* is a keystone species in must precisely because its physiology is better matched to the must environment than physiologies of other fungi.

We experimentally confirmed that *S. cerevisiae* is a keystone species by reintroducing it to low-functioning D7 microcosm communities. Low-functioning microcosm communities 2, 4, and 8 all became high-functioning when *S. cerevisiae* was added, and high-functioning microcosm community 1 remained high-functioning when *Nakazawaea*, *Cryptococcus*, or *Hanseniaspora* isolates were added (Fig. 5, S9). In contrast, while *Nakazawaea* completely consumed glucose when grown alone, and adding *Nakazawaea* to a community had non-additive effects on glucose consumption, its presence did not cause microcosms to completely consume glucose. Both *Nakazawaea* and *Cryptococcus* species had non-additive effects on community glucose consumption, and therefore fit our definition of keystone species in at least some microcosm contexts. *S. cerevisiae* had higher keystone indices than either, with the possible exception of *Cryptococcus* in microcosm 8, which had marginally significantly different keystone indices from *S. cerevisiae*. While *S. cerevisiae* behaves as a keystone species more than other yeasts from the must used in this study, it may not be the only keystone must species globally. Further research is needed to confirm *S. cerevisiae* as a keystone species in communities containing other highly fermentative yeasts, such as *Dekkera bruxellensis* (syn. *Brettanomyces bruxellensis*) and *Schizosaccharomyces pombe* (Hagman et al., 2013).

Saccharomyces inhibits other microorganisms through a variety of mechanisms, and this inhibition likely contributes to its behaviour as a dominant and keystone species. *Saccharomyces* cultures produce large quantities of ethanol, which poisons ethanol sensitive microbes (Goddard, 2008). Ethanol is not the only inhibitory molecule produced by *Saccharomyces*, however; it also produces secreted toxic proteins or glycoproteins ('killer toxins'), short chain fatty acids, and sulphur dioxide, all of which can be toxic to other microbes (Fleet, 2003; Albergaria et al., 2010). It can also directly inhibit some yeast species through cell-cell contact (Nissen and Arneborg, 2003). Finally, *Saccharomyces* has a high growth rate, and can limit slower growing competitors by reducing nutrient concentrations as it grows (MacLean and Gudelj, 2006).

Despite the high functional impact of *S. cerevisiae* presence, some *S. cerevisiae* sequences were present in low-functioning D7 microcosms. We suspect that the dilution treatment completely removed *S. cerevisiae* cells from low-functioning D7 microcosms, and that detected sequences originated from sequencing errors, extracellular DNA, or dead cells. Alternatively, *S. cerevisiae* may have been present in low-functioning D7 communities, but early stochastic changes in relative organism frequencies prevented it from becoming dominant. We attempted to minimize sequencing errors by removing singleton OTUs and sequences predicted to be chimeric: both result from errors during PCR amplification and are unlikely to represent sequences present in the community (Dickie, 2010; Tedersoo et al., 2010). Additionally, sequences with incompatible barcodes on their 5′ and 3′ ends were assumed to result from tag-switching during or after PCR and discarded (Carlsen et al., 2012). Nevertheless, sequencing errors cannot be avoided; such errors were present in our constructed control communities, which were biased towards detecting *S. cerevisiae* sequences (Fig. S3). Constructed control communities also showed higher sequenced species richness than expected, due either to unaccounted for sequencing errors (*e.g.,* cross-contamination during PCR and library preparation, undetected PCR errors), OTU clustering errors, or the presence of extracellular DNA in the grape must substrate. Cells that died over the course of microcosm incubation may also have been detected in low-functioning D7 communities because PCR can detect DNA for weeks after cell death (Josephson et al., 1993). We complemented DNA sequencing data with culturing data in D7 microcosms to help account for potential errors. If *S. cerevisiae* was indeed present in low-functioning D7 microcosms, it represented less than 5% of total culturable cells at the end of incubation.

4.2. Limitations to S. cerevisiae dominance at high diversity

Glucose consumption and ending *S. cerevisiae* frequency were also low in D0 microcosms (Figs. 3 and 4). Results from D7 microcosms and artificially assembled communities suggest a causal relationship between low *S. cerevisiae* dominance and low glucose consumption. We speculate that rare species present in D0 microcosms, but absent from other microcosms, indirectly prevented complete glucose consumption by preventing *S. cerevisiae* dominance.

Antagonistic interactions with rare species most likely depressed *S. cerevisiae* growth early in succession, preventing *S. cerevisiae* from reaching the frequency needed to achieve dominance (Fig. 3). Wine yeasts can negatively influence *Saccharomyces* growth through both interference and exploitative competition. Toxic compounds produced by non-*Saccharomyces* yeasts produce include killer toxins and toxic fatty acids, which can kill or inhibit *Saccharomyces* (Michalčáková et al., 1993; Bisson, 1999). Non-*Saccharomyces* yeasts can also reduce nutrient concentrations in fermenting must, most notably thiamine, or reduce must pH, resulting in slow *Saccharomyces* growth (Bisson, 1999). While we did not measure bacterial diversity, antagonistic bacteria can also reduce substrate pH or produce toxic compounds and inhibit *Saccharomyces* growth (Magnusson and Schnürer, 2001; Fleet, 2003; Muthaiyan et al., 2011).

Although antagonistic interactions with rare species are a likely cause of low *S. cerevisiae* frequency in D0 communities, there are two alternatives: the presence of grape solids in microcosms, or high starting biomass, could have influenced *S. cerevisiae* frequency or ecosystem function. We did not remove grape solids from microcosms because we wanted to include all possible sources of rare species inoculum; as part of our dilution treatments, grape solids were introduced in small amounts to all microcosms. We also did not normalize starting biomass by regrowing dilute inocula before inoculating microcosms, as other studies have (*e.g.,* Hernandez-Raquet et al., 2013), because regrowing inocula would have resulted in decreased species richness (Figs. 1 and 2). However, we expect all microcosms to have reached the maximum densities possible under experimental conditions. When *Saccharomyces* is present, we expect maximum densities to be the same regardless of inoculum size because high and low inoculum sizes produced similar densities of *Saccharomyces* monocultures (Fig. S10). Unfortunately, there is no way to completely disentangle the effects of inoculum size with species richness in an investigation that includes rare and unculturable species in the natural must

community. Of the possible explanations for low final *S. cerevisiae* frequency and glucose consumption in microcosms, we consider antagonism by rare species to be most likely.

All microcosms were shaken during incubation, and oxygen availability may have given *S. cerevisiae* a competitive disadvantage in D0 microcosms. *S. cerevisiae* is a facultative anaerobe, and usually ferments sugars in the presence of oxygen (Visser et al., 1990; Pronk et al., 1996). But many grape-associated yeasts are obligate aerobes and cannot ferment sugars, while other fermenting yeasts have competitive disadvantages relative to *S. cerevisiae* at low oxygen concentrations (Visser et al., 1990; Setati et al., 2012). Grapes and grape plants contain diverse fungal communities (Barata et al., 2012; Pancher et al., 2012), and many of these plant-associated taxa were present in both our inoculum and early succession winery vats (Figs S5-S7). While plant-associated fungi often influence wine quality by altering the must substrate, they generally do not persist in fermentations because they are limited by low oxygen concentrations (Pardo et al., 1989; Barata et al., 2012). In contrast, our microcosm conditions permitted growth of obligate aerobes. Fermenting organisms frequently outcompete respiring organisms because they have high relative growth rates, but competition in D0 microcosms may be mediated by antagonistic interactions instead of resource competition (Pfeiffer et al., 2001; Nissen and Arneborg, 2003). Rare aerobic fungi that would otherwise be absent from oxygen-limited fermentation vats may engage in antagonistic interactions to kill or prevent growth of *S. cerevisiae*.

While *S. cerevisiae* often ferments glucose when oxygen is present, fermentation is a less efficient cellular process than respiration (Hagman et al., 2013). Therefore, we would expect microcosms with high *S. cerevisiae* frequency to have relatively low biomass. Instead, increasing *S. cerevisiae* frequency weakly correlated with increasing biomass (Fig. S8b). While we do not know why high frequency of a fermentative organism would lead to high relative biomass, we consider two possibilities. Competing must organisms may be engaging in costly secondary metabolism, including producing chemicals that inhibit *S. cerevisiae* growth at the expense of biomass production. At the same time, *S. cerevisiae* may be regaining much of the energetic cost of fermentation by aerobically respiring ethanol, a by-product of fermentation (Piškur et al., 2006). Inefficient fermentation could lead to high *S. cerevisiae* biomass relative to competing organisms when the costs of secondary metabolism or benefits of ethanol respiration are high, but further work is needed to confirm these mechanisms.

Gas exchange in microcosms may also have prevented *S. cerevisiae* in D0 microcosms from poisoning other yeasts with ethanol for a competitive advantage. Under winemaking conditions, *S. cerevisiae* ethanol production may exclude potential competitors (Goddard, 2008). But in D0 microcosms, aerobic ethanol respiration by *S. cerevisiae* or ethanol evaporation could potentially permit growth of rare species that would otherwise be excluded. Ethanol poisoning is a controversial hypothesis to explain *S. cerevisiae* dominance because some common wine yeasts are as ethanol tolerant as *S. cerevisiae* (Pina et al., 2004; Goddard, 2008). But rare species may be rare precisely because they are ethanol sensitive. As with aerobic yeasts, ethanol-sensitive yeasts may inhibit or kill *S. cerevisiae*. If antagonistic aerobic or ethanol-sensitive competitors are rare, we expect them to be present in D0 microcosms but diluted from other microcosm treatments.

The influence of gas exchange may have given *S. cerevisiae* a competitive disadvantage in D0 microcosms even though neither *S. cerevisiae* dominance nor sugar consumption were inhibited in winery vats. Both the must substrates and microbial inocula were nearly identical in microcosms and vats (vats may have had higher overall species richness because of their large volumes). *S. cerevisiae* frequency increased in vats over time (Fig. S4) and the microbial community completely consumed available sugars (97.2–99.5% total sugars consumed). The winemakers calculated vat sugar concentrations using must density and temperature (Saracco and Raffo, 1990). Abiotic environments did differ between microcosms and vats: microcosms were millilitres in volume, held at a constant temperature, and continuously shaken, while vats were hectolitres in volume, permitted to change in temperature (range of 17–33 °C, measured by the winemakers), and mixed about once daily. Increased gas exchange likely permitted antagonistic aerobic or ethanol sensitive species that would not survive the vat environment to outcompete *S. cerevisiae* in D0 microcosms.

4.3. Microbial richness-function relationships

Richness may have indirectly influenced must ecological function by permitting or preventing *S. cerevisiae* dominance, resulting in low function when *S. cerevisiae* does not reach high frequencies (Figs. 3 and 4). At high richness, antagonistic interactions most likely prevented *S. cerevisiae* from becoming numerically dominant; at low richness, our treatments diluted *S. cerevisiae* from grape must; and at intermediate richness, *S. cerevisiae* dominated grape must and drove ecosystem function. Dominance is not the only possible mechanism producing nonmonotonic relationships between richness and function: in some fungal decomposer communities, hump-shaped curves similar to the one observed in must result from interactions between antagonism and facilitation, or antagonism and niche complementarity (Toljander et al., 2006; Costantini and Rossi, 2010). Dominance may play a smaller role in decomposer communities than in must communities because costly antagonistic interactions in species-rich decomposer communities can lead to inefficient resource use (Boddy, 2000; Toljander et al., 2006).

In contrast, *S. cerevisiae*'s high fermentation rate may give it a competitive advantage and lead both to high ecosystem functioning and numerical dominance in communities with intermediate richness (De Deken, 1966; Visser et al., 1990; MacLean and Gudelj, 2006; Goddard, 2008). Dominant species' presence correlatse with species richness in some other heterotrophic communities, while they impact function more than richness alone (Dangles and Malmqvist, 2004). Dominant species can also decrease species richness while driving high ecosystem function, as in grape must and some shredder communities (Creed et al., 2009). Dominance may have consistently strong effects on function in systems that, like grape must, lose richness as a species becomes dominant. Such systems include ephemeral habitats, habitats undergoing disturbance, and human diseases, especially diseases resulting from changes in human microbiome communities (Fish and Hall, 1978; Foster and Gross, 1998; Delhaes et al., 2012; Sladecek et al., 2013).

Grape must communities may be particularly prone to both ecological dominance and species loss over time because they are highly disturbed communities. Must is characterised by a sudden change in the structure of the grape substrate and a release of large quantities of sugar. Such disturbances and resource pulses can lead to high invasibility in microbial communities, and may have contributed to *S. cerevisiae* dominance (Liu et al., 2012; Mallon et al., 2014). *S. cerevisiae* is also the most abundant fungal species on many winery surfaces before grapes are harvested; its abundance on winery surfaces may allow it to quickly inoculate freshly available grape must (Bokulich et al., 2013). In contrast, many other early must species may not be adapted to the grape or winery

environments because must communities suddenly assemble from disparate microbial communities (*e.g.,* grape, vector, and winery microbes) every year when grapes are harvested and processed. Loss of poorly adapted species early in fermentation most likely accounts for much of the observed decrease in species richness in winery vats over time. It is unlikely that all community members are maladapted to grapes or must, despite both habitats being ephemeral. Many wine microbes, including *S. cerevisiae*, may be adapted to dormancy or habitat switching when fruit substrates are not available (Stefanini et al., 2012). We also observed *Hanseniaspora*, *Nakazawaea*, and *Cryptococcus* isolates in must; these isolates were not selected out of the must environment after more than 14 d had passed. On the other hand, while *S. cerevisiae* is dominant in must and winery surfaces, it is rare on grapes. D0 microcosms may have contained rare grape-adapted fungi that outcompete *S. cerevisiae* when oxygen is abundant, but are poorly adapted to oxygen-limited conditions in fermentation vats.

5. Conclusions

S. cerevisiae is a keystone species whose presence drives wine must ecosystem function. Changes in species richness had different impacts on *S. cerevisiae* dominance in experimental microcosms, and consequently on ecosystem function, in species-rich and species-poor communities. Varying impacts of richness on *S. cerevisiae* dominance most likely led to an unusual richness-function relationship: ecosystem function increased, then decreased, as inoculum size increased. These results may be generalizable to other communities with dominant or keystone species, especially when the mechanisms leading to numerical dominance also contribute to ecosystem function. Our results highlight the importance of investigating the entire possible range of species richnesses in a community. Microbial communities can be much more species-rich than plant and animal communities (Blackwell, 2011), and ecosystem function can respond to changes in richness differently in hyperdiverse and depauperate communities.

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Supplementary data

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