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Ecology and Evolutionary Biology as Frameworks to Study Wine Fermentations

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ABSTRACT

Winemaking has leveraged microbiology to enhance wine quality, typically by engineering and inoculating individual yeast strains with desirable traits. However, yeast strains do not grow alone during wine fermentation, rather they are embedded in diverse and evolving microbial communities exhibiting complex ecological dynamics. Understanding and predicting the interplay between the yeast community over the course of the species succession and the chemical matrix of wine can benefit from recognising that wine, like all microbial ecosystems, is subject to general ecological and evolutionary rules. In this piece, we outline how conceptual and methodological frameworks from community ecology and evolutionary biology can assist wine yeast researchers in improving wine fermentation processes by understanding the mechanisms governing population dynamics, predicting and engineering these important microcosms, and unlocking the genetic potential for wine strain development.

1 | Introduction

For centuries, humans have refined winemaking through the sciences of viticulture and oenology. Traditionally, these fields have advanced separately, developing distinct bodies of technical knowledge to first efficiently grow grapes and then produce high-quality wines. Following the discovery of the biological basis of fermentation (Pasteur 1860), the model yeast species *Saccharomyces cerevisiae* have been at the forefront of wine research. This research has largely adopted a reductionist approach, focusing on understanding and efficiently controlling *S. cerevisiae* metabolism in axenic conditions (Gonzalez and Morales 2022). In recent years, the many non-*Saccharomyces* yeasts species on the grape microbiome have increasingly been recognised as a key element connecting the vineyard ecosystem with the fermentation processes in the winery (Belda et al. 2017).

Microbial communities established in grape surfaces serve as the source for those microbes that will participate in the fermentation processes (Barata et al. 2012). Filamentous fungi are abundant in the grape microbiome and play a critical role in grape quality, as they can cause several grape rots (i.e., *Botrytis, Mildiu, Oidium* genera). Apart from the direct effect on grape quality, filamentous fungi will shape the structure of yeast and bacterial communities that will be found in fresh grape musts. Following grape crushing, most filamentous fungi will disappear and the potentially active fraction of the grape microbiome will be reduced to osmophilic and fermentative yeasts, and lactic acid and acetic acid bacteria, that engage in complex ecological and metabolic interactions

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during fermentation. Traditionally, the presence and activity of non-*Saccharomyces* yeasts on grapes was seen as sources of wine spoilage, prompting winemakers to add sulphites to grape musts and to inoculate high doses of selected *S. cerevisiae* strains to rapidly displace and dominate over native yeast communities (Jolly, Varela, and Pretorius 2014). Although this approach offers advantages in terms of fermentation kinetics and reproducibility, it often results in the over-standardisation and simplification of the wine's chemical profile compared with spontaneous fermentations (Rainieri and Pretorius 2000). To enhance wine quality, microbiologists have developed two alternatives: (i) manipulating the outcome of spontaneous fermentations by precisely controlling environmental factors (pH, temperature, nutrient availability, etc.); or (ii) designing complex yeast consortia that include a mix of yeast species with enhanced functionality over *S. cerevisiae* monocultures (Ciani et al. 2010). The different yeast species in these communities generate and consume different metabolites in diverse fermentation conditions and thus can shape wine quality both positively and negatively in different contexts. Understanding how abiotic (physical–chemical parameters of grape musts) and biotic factors (interspecies interactions) and their interdependence influence the fermentation performance of yeast communities has thus become the central aim of wine research.

Numerous studies have described microbiome patterns in vineyards and report the impact of specific yeast strains on wine fermentation performance (Belda et al. 2017; Bokulich et al. 2014, 2016; de Celis et al. 2024; Knight et al. 2015; Ramirez et al. 2020). These contributions have successfully addressed some technical challenges and provide substantial insights into the microbial features of the winemaking system. However, they remain largely descriptive and rarely set out to fully characterise the ecological and evolutionary processes occurring within the wine ecosystem or the underlying molecular mechanisms that govern them (Conacher et al. 2021). Most studies in wine yeast research lack clear hypothesis testing, resulting in the accumulation of specific observations rather than the generation of fundamental and generalisable knowledge (Prosser 2020). To move beyond simple descriptions of **microbial consortia** to the precise management of multi-species yeast communities, we argue that applying the conceptual framework of classical ecological theory (Marquet et al. 2014) and modern eco-evolutionary approaches (Loreau, Jarne, and Martiny 2023), will be essential.

Recognising that wine fermentation is governed by the same ecological and evolutionary principles as other natural ecosystems opens up the opportunity to apply the wealth of ecology and evolutionary theory that has been developed over the past century. We suggest that bringing wine researchers closer to the vocabulary (see Box 1), theoretical foundations and research methods of ecologists and evolutionary biologists can yield qualitative and quantitative advances in our current understanding of wine fermentations. Moreover, we argue that 'ecology and evolution have as much to contribute to wine as wine has to contribute to ecology and evolution', so reconciling the interests of these disciplines will have bidirectional benefit. Introducing fundamental eco-evolutionary concepts in the context of wine yeast communities and presenting the ecological significance of wine fermentations is the first step and what inspires us to address this with the simultaneous writing of this work and its companion piece in Environmental Microbiology (Belda et al. 2025).

2 | Community Ecology as a Framework to Study Wine Fermentations

Microbial community ecology studies the organisation and the functioning of multi-species microbial assemblages across spatial and temporal gradients (Konopka 2009). Prosser and Martiny (2020) identify four primary questions that can be addressed through microbial community ecology: (i) how eco-evolutionary processes drive microbial community assembly, (ii) how community composition responds to environmental changes, (iii) how microbes interact within the community and (iv) how to predict ecosystem functions based on community structure. In this first section, we will introduce key concepts from community ecology theory that allow one to explore and address these questions in the context of wine fermentation.

Understanding how different species assemble into microbial communities is a central pursuit in community ecology. **Community assembly** theory posits that species assemble non-randomly, influenced by various ecological processes such as dispersal, environmental filtering and biological interactions (Soberon and Peterson 2005). The wine fermentation ecosystem provides an intriguing scenario to study these processes across different spatial scales. At a broad scale, a large body of research has focused on the biogeographical patterns that explain the distribution of microbial biodiversity on soil, vines and grape surfaces in vineyards, and how this diversity serves as a reservoir for the subsequent fermenting musts in the winery (Gobbi et al. 2022; Griggs et al. 2021; Morrison-Whittle and Goddard 2018; Onetto et al. 2024). For example, a recent hypothesis-driven study by Jiraska et al. (2023) demonstrated that ecological habitat (i.e., soil vs. plant) significantly influences community composition more than vineyard location or farming management regime. They found no consistent decrease in community similarity with greater spatial distance within sites, suggesting that vineyards function as discrete biodiversity islands with a characteristic species pool seeding fermentation.

The microbial communities that have assembled on grape surfaces in vineyards will arrive at winery facilities and serve as a natural inoculum for wine fermentation. Following grape crushing, which transfers microbial communities from a solid to a liquid environment, the ecology of wine fermentation is initially governed by strong **environmental filtering** (Figure 1), as fungal species respond to the high osmolarity and low pH found in grape musts (Barata et al. 2012). Decreasing oxygen availability and, in most cases, the presence of sulphites added to fresh grape must also play important roles, representing examples of environmental factors that can be deliberately modified by winemakers and which have major implications on the initial yeast community composition. For example, addition of oxygen to wine by aeration has been shown to promote the presence of some fermentative non-*Saccharomyces* yeast species, such as *Torulaspora delbrueckii* and *Lachancea thermotolerans* (Hansen et al. 2001).

Following the initial environmental filtering after grape crushing, there is a temporal window in which wine fermentation exhibits high ecological complexity due to the wide variety of different nutrients available (carbon, nitrogen and vitamin sources). This complexity leads to the growth of multiple different yeast species that exploit different niches (Figure 1). Although *S. cerevisiae*

BOX 1 | Glossary of terms which matters for understanding wine-related ecological and evolutionary processes.

Microbial consortia: Associations of free-living microorganisms in which the members interact in close proximity with one another. (Cao et al. 2023). These associations often enable the community to perform complex biological processes that individual species may not accomplish alone.

Microbial Community Assembly: Process by which different microbial species come together to form a community and establish networks of interactions. These interactions can depend on mechanisms such as physical adhesion, biofilm formation, trophic interactions, signalling and horizontal gene transfer (Cao et al. 2023).

Pairwise Interactions: Pairs of microbial species will establish interactions with each other when co-occurring and these can be classified according to the effects on each partner in the interaction (positive, neutral or negative). These categories include competition, amensalism, commensalism, mutualism, symbiosis and predation (See Meroz, Livny, and Friedman 2024 for a review).

High-order interactions: Interactions among three or more microbial species, where the presence or activity of one species alters the interactions between others, leading to emergent effects that cannot be predicted from species pairs (Grilli et al. 2017; Sanchez 2019).

Priority effect: The impact of species arrival order, timing and relative abundance on ecosystem community structure and assembly (Chappell et al. 2022).

Environmental filtering: Abiotic factors limiting or selecting the species distribution and abundance in a certain habitat. This process interplays with biotic interactions resulting in a certain community assembly, but it does not account for interactions among organisms on its own (Cadotte and Tucker 2017; Kraft et al. 2015).

Bottom-up assembly: The process of assembling new microbial consortia by combining individual strains that have previously been isolated. This approach allows for the controlled design and manipulation of microbial communities, enabling researchers to study specific interactions and engineer consortia for applications in synthetic ecology, biotechnology and environmental processes.

Top-down assembly: A microbial consortia assembly strategy that relies on selecting a subset of species from a naturally isolated community. This approach typically involves modifying or simplifying a complex, natural community by selecting species on the basis of their functional traits or interactions, allowing researchers to maintain ecological relevance while optimising the consortia for specific applications (Sanchez et al. 2021).

Dilution-to-extinction: A method that progressively dilutes microbial communities until only a few species remain. It can be used to study simplified microbial subsamples that preserve community function (Sanchez et al. 2021).

Ecological invasion: Entry of a new species to a foreign ecosystem or ecological niche. Invasions can lead to community instability and changes in community composition and function (Mallon, Elsas, and Salles 2015; Vila et al. 2019).

Coalescence: The process by which different microbial communities are mixed and reassembled to form a new community. It may involve changes in the community composition and functions. Examples of coalescence are frequent in the microbial context, such as the leaves' microbiota contacting the soil microbiome during fall (Rillig et al. 2015) or in clinical applications like in faecal microbiota transplants (Li et al. 2016).

Adaptive Laboratory Evolution: A technique for driving the evolution of microorganisms, allowing for the selection of strains with enhanced characteristics from the evolving population. This is achieved by artificially replicating the processes of mutation and selection that occur in natural environments, under controlled laboratory conditions (Wang et al. 2023).

Artificial Ecosystem Selection: The application of artificial selection techniques used to select for specific traits in organisms of interest to enhance functions of interest in whole ecosystems or communities (Swenson, Wilson, and Elias 2000). This approach relies on the existence of traits that display heritable variation at the community level (Chang et al. 2023; Goodnight 2000). Also referred to as directed evolution of microbial communities (Sanchez et al. 2021).

specialises in growing in nutrient-rich environments—exploiting specific nutrient sources (i.e., hexoses and ammonium as carbon and nitrogen sources, respectively)—and engineering the environment through fermentation (Goddard 2008), several studies have found that different wine yeast strains exhibit varying preferences for nitrogen sources available in grape musts (Gobert et al. 2017). For example, in a recent study, we found that many non-*Saccharomyces* yeast species can more efficiently utilise alternative nutrient sources, such as organic acids and some amino acids, than *S. cerevisiae* (Figure 2A), and thus perform better under fermentation conditions with limited vitamin and nitrogen concentrations (Figure 2B) (Data adapted from Ruiz et al. 2023). A detailed characterisation of all the exploitable metabolic niches in wine fermentations, in terms of their ecological significance, temporal stability and the species capable of occupying them, is essential for deciphering the functional roles of wine yeast species (i.e., production of metabolite of interest), and for predicting population dynamics.

Several studies have demonstrated the utility of predictive models, not only for advancing the understanding of the metabolic and physiological mechanisms underlying wine fermentation but also for enhancing this industrial process (Petruzzi et al. 2022). Henriques et al. (2018) employed dynamic modelling to analyse key fermentation mechanisms in *S. cerevisiae* and *Saccharomyces kudriavzevii*, revealing differences in

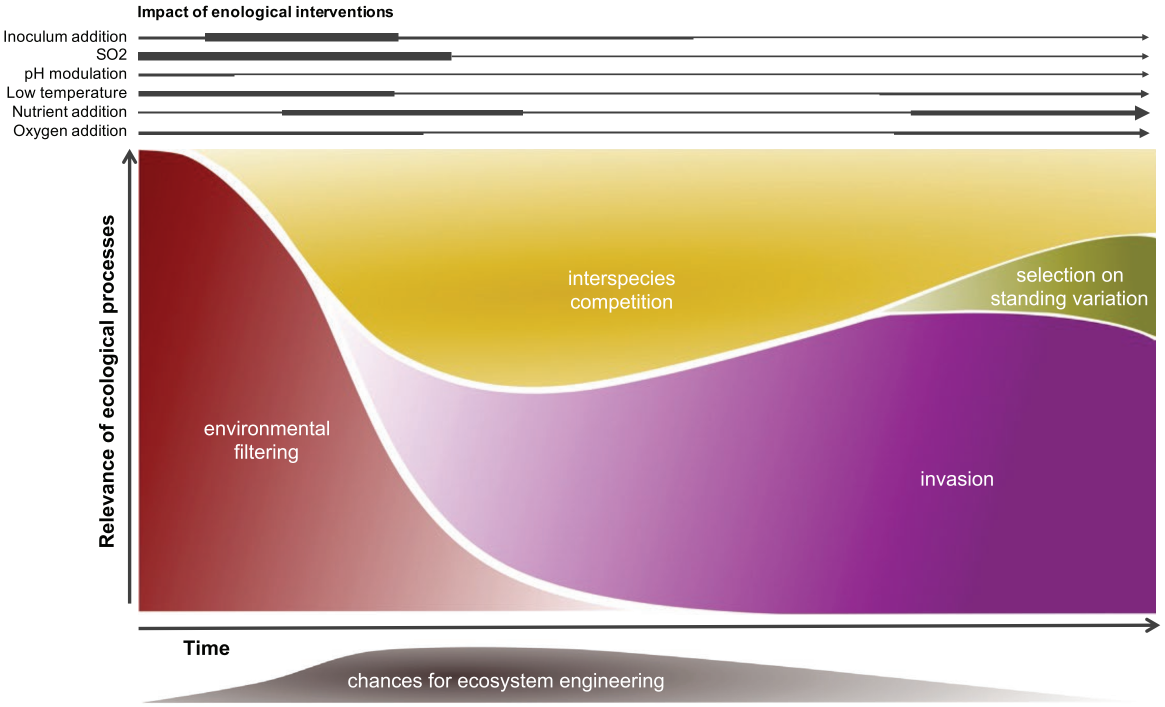

FIGURE 1 | Understanding the wine fermentation from an ecological perspective can provide insights about the biological dynamic within it and how these can be manipulated to achieve desired fermentation outcomes. Four main ecological and evolutionary processes drive population dynamics within a single wine fermentation, each holding varying levels of importance at different stages. Initially, environmental filtering—shaped by the abiotic characteristics of wine fermentation (such as high osmolarity and low pH)—dominates. Following this, interspecific interactions become the dominant ecological process governing community dynamics. At this point, as ecological complexity increases, so do the opportunities for ecosystem transformation through natural processes like niche construction and artificial oenological interventions, such as environmental modification (e.g., sulphite addition or nutrient supplementation) and yeast species inoculation. These ecological processes culminate in the establishment of a moderately diverse community of fermenting yeasts, led by the dominance of *S. cerevisiae* strain populations, in a process that mirrors some key aspects of ecological invasions. Even as this keystone species comes to dominate the community different strains will continue to compete with and displace one another as the *S. cerevisiae* population adapts to the wine environment via selection on genetic variation.

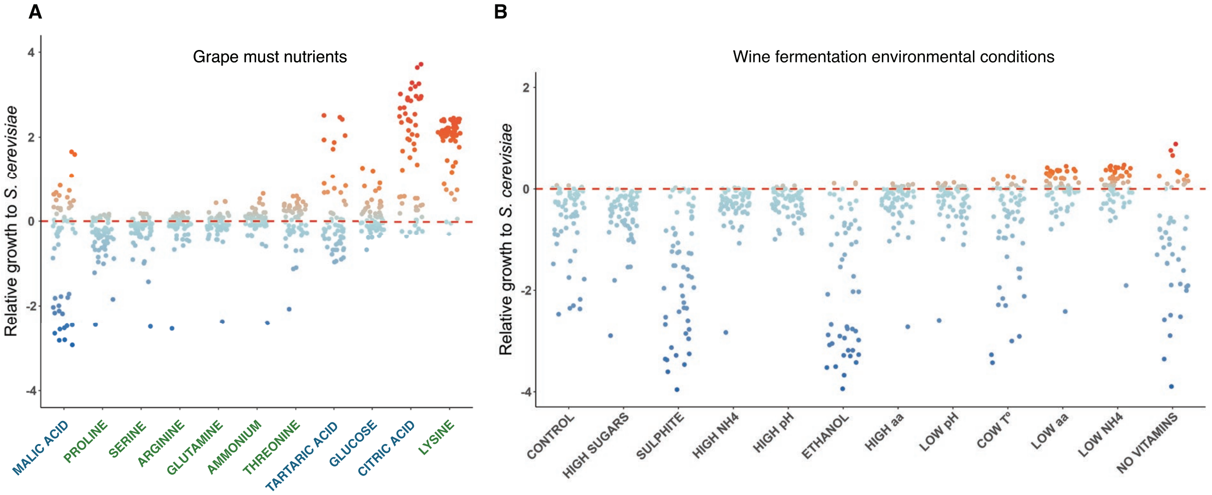

FIGURE 2 | Some non-*Saccharomyces* species exhibit a better performance than *S. cerevisiae* using specific nutrients (carbon sources in blue and nitrogen sources in green) found in grape must and under specific conditions within the wine fermentation ecosystem. Here, we show data of the growth rate of 60 strains, belonging to 30 different species assayed in a set of growth conditions, compared with that of *S. cerevisiae*. Each dot corresponds to an individual value and the colour scale represents how better (in red) or worse (in blue) a strain grow compared with *S. cerevisiae*. Data adapted from Ruiz et al. (2023).

metabolic flux distribution between the two species and highlighting the potential of predictive model for optimising wine quality in mixed fermentations. More recently, Moimenta et al. (2023) developed a mechanistic model incorporating yeast physiological states to describe biomass production and secondary metabolite dynamics during fermentation, demonstrating its value in optimising process design and influencing aromatic profiles through nitrogen management. As the ecological complexity increases during the early stage of fermentation, so does the potential complexity of interspecies and species–environment interactions (Figure 1). Although a large body of empirical work has mapped the intricate network of yeast interactions that govern wine fermentation, relatively few studies have systematically characterised the mechanistic basis of these interactions (Albergaria and Arneborg 2016; Bagheri et al. 2020; Ciani et al. 2016; Lax and Gore 2023; Planells-Cárcel et al. 2024; Pourcelot et al. 2024; Roullier-Gall et al. 2022; Ruiz et al. 2023). In addition to the competition for limiting nutrients that we described previously, known mechanisms of interaction during the early stages of wine fermentation included the following: the production of antimicrobial molecules (e.g., killer toxins or pulcherriminic acid), contact-dependent interactions mediated by flocculins, the production of *quorum* sensing molecules (Mencher et al. 2021), and the production of extracellular vesicles, as recently demonstrated by Morales et al. (2021). Yeast growth during the early stages of wine fermentation can thus modify the environment in a wide range of manners, both by releasing new exploitable resources and generating additional abiotic stressors. We are still far from understanding the complex feedbacks between these different mechanisms of interaction and elucidating them remains an ongoing area of research.

Most research on the microbial contribution to wine fermentation has traditionally focused on yeasts. Some studies, however, have highlighted the significant role of bacteria, particularly lactic acid bacteria and acetic acid bacteria, in shaping the final properties of wine and influencing the ecological processes driving fermentation dynamics. An illustrative example is provided by Ponomarova et al. (2017), who demonstrated a mutualism phenomenon between *S. cerevisiae* and *Lactococcus lactis*, in which *S. cerevisiae* enriches the nitrogen content of grape must by secreting amino acids, whereas *L. lactis* releases free sugars through lactose catabolism. Building upon this, Balmaseda et al. (2024) revealed that the lactic acid bacterium *Oenococcus oeni* benefits from peptide enrichment mediated by *T. delbrueckii* during alcoholic fermentation, resulting in a shorter malolactic fermentation period.

3 | Top-Down versus Bottom-Up Approaches to Engineering Microbial Ecosystems

Multi-species microbial consortia can be designed and manipulated to deliver community-level processes that maximise ecosystem performance during fermentation (i.e., improving fermentation kinetics and producing desired metabolites). Two complementary approaches have been developed for optimising the functions of multi-species communities. **Bottom-up assembly** approaches begin with individual strains or species and seek to identify the optimal combinations to perform a specific function. For instance, one could start with a set of wine yeast species that each exhibit desirable traits (such as producing aroma-related compounds) and then map out how different yeast combinations interact—both qualitatively and quantitatively—to impact that trait (Meroz, Livny, and Friedman 2024). While mapping all the **pairwise interaction** may be sufficient for identifying optimal combinations of species in some cases (Friedman, Higgins, and Gore 2017), multiple studies have shown that **higher-order interactions** govern the functional contributions of individual species, making pairwise co-culture predictions insufficient (Sanchez-Gorostiaga et al. 2019). Indeed, several recent studies confirm that higher-order interactions are widespread within wine yeast ecosystems (Conacher et al. 2022; Ruiz et al. 2023). Despite their complexity, a recent study by Diaz-Colunga et al. (2024) developed a framework to predict ecosystem functions without needing to assemble all possible species combinations—an endeavour that would result in an astronomically large combinatorial space. In multi-species communities, widespread species-by-species interactions lead to emergent species-by-community interactions that are well-captured by simple linear regression models (Diaz-Colunga et al. 2024; Skwara et al. 2023). Thus, although bottom-up approaches typically require prior metabolic characterisation of strains to combine them rationally, recent evidence shows that the performance of multi-species consortia can be predicted on the basis of fundamental ecological traits of the constituent taxa (Giri, Shitut, and Kost 2020; Sanchez et al. 2023). In our recent study, we demonstrated this approach by successfully predicting sugar consumption in multi-species wine-fermenting communities assembled from random combinations of up to 10 species (Ruiz et al. 2023), suggesting that other important properties of wine fermentation may also be predictable using this framework.

Top-down assembly approaches to microbial consortium engineering address the issue of optimising ecosystem function from an orthogonal direction starting with an existing natural community and then manipulating this community to achieve a desired output (Sanchez et al. 2021). For example, through modification of the wine fermentation environment (e.g., cold pre-fermentation maceration, sulphite addition or oxygen management), oenologists have been able to steer the composition and function of wine yeast communities (Benucci et al. 2018). As another example, the traditional method of *coupages* (here applied to the practice of fermenting grape musts from different vineyard blocks and varieties as a blend in the same fermentation tank) also represents a form of top-down engineering as different microbial communities are mixed (**coalescence**) leading to higher community diversity. An as of yet unexplored top-down approach for engineering wine yeast consortia is **Artificial Ecosystem Selection** (Sanchez et al. 2021; Swenson, Wilson, and Elias 2000). Artificial Ecosystem Selection seeks to take the breeding techniques that were historically used to select for desirable traits in agricultural crops and animals and extends them to select for desirable traits in multi-species communities (Swenson, Wilson, and Elias 2000). To date, most of the work exploring Artificial Ecosystem Selection approaches has been theoretical (Chang et al. 2023; Doulcier et al. 2020), though recently, Arias-Sánchez et al. (2024) used this approach to improve pollutant degradation by bacterial communities. These approaches may be particularly suitable for engineering wine-fermenting consortia as they do not require prior knowledge of the mechanisms responsible for the desired function and already take into account the natural ecological interactions within the ecosystem.

4 | *S. cerevisiae* as an Invasive Species

In the previous sections, we focused on the earlier stages of wine fermentation and in particular on the role of non-*Saccharomyces* wine yeast species. Successful fermentation ultimately relies on the eventual takeover of *S. cerevisiae* on a reasonable timescale as only *S. cerevisiae* is able to tolerate the high ethanol concentrations that are generated by complete sugar fermentation (Jolly, Varela, and Pretorius 2014). Ensuring the successful takeover of *S. cerevisiae* is thus crucial for oenologists to prevent stuck fermentations and prevent organoleptic deviations. Understanding the ecological and evolutionary mechanisms that either prevent or facilitate the development of *S. cerevisiae* populations is, therefore, of significant industrial importance. Because *S. cerevisiae* is found in very low numbers in the vineyard microbiome, and also as a minority species in fresh grape musts, we propose that its takeover during wine fermentation can be seen as an invasion and can thus be understood through the lens of **ecological invasion** theory (Figure 1).

Invasion ecology teaches us that invasion success depends on both the ecological features of the invader species and those of the native community (Kinnunen et al. 2016). On the invader side, there first has to be a sufficient number of cells of *S. cerevisiae* (propagule pressure) to overcome the effect of ecological drift (stochastic extinction) (Acosta et al. 2015; Vila et al. 2019). The most commonly used approach for achieving a high initial propagule pressure is the addition of commercial starters of *S. cerevisiae*, although this comes at the cost of reduced sensory complexity (Rainieri and Pretorius 2000). An alternative approach is to use a '*pied de cuve*' in which a starter culture is prepared by fermenting a small volume of grape must a couple days before the main fermentation in an attempt to enrich for *S. cerevisiae* (Börlin et al. 2020). The starter culture is then added to the main barrel after harvesting, increasing the initial dose of *S. cerevisiae*.

Even if introduced at a high enough initial density, *S. cerevisiae* must be able to outcompete other community members. Although *S. cerevisiae* is extremely efficient at consuming sugars, wine fermentation is nitrogen-limited and in vitro experiments have shown that many non-*Saccharomyces* species can outcompete *S. cerevisiae* when *S. cerevisiae* is introduced at lower initial abundance, highlighting the role of **priority effects** (Chappell et al. 2022). In a recent study, Lax & Gore showed that pairwise interactions between *S. cerevisiae* and non-*Saccharomyces* were density dependent at low ethanol concentration, but that increasing ethanol concentration tilts the balance to *S. cerevisiae* (Lax and Gore 2023). This suggests that ethanol production by *S. cerevisiae* may serve as an ecological weapon allowing it to invade and displace a wine fermentation community even when all the nutrient niches have been occupied.

Alternatively, the wine industry aims to find the ecological features of microbial communities that are easily invaded by *S. cerevisiae*. Thus, in addition to the invader, the properties of the native community can also influence invasion outcome. Firstly, more species-rich communities tend to be more invasion resistant because of increased competition and/or niche packing (Tilman 2004; Vila et al. 2019). For example, Boynton & Greig performed a dilution-to-extinction experiment and found that high diversity grape must communities tended to inhibit takeover of *S. cerevisiae* and resulted in stuck fermentation. We note however that resistance-rich relationships are not always that clear and can even change its direction in different abiotic contexts. For example, in the dilution-to-extinction experiments described previously, shaking may have put the *S. cerevisiae* at a competitive disadvantage by increasing oxygen concentration and preventing/minimising the amount of ethanol production (Boynton and Greig 2016).

Secondly, the similarity of the invaders and the native community can also significantly influence the invasion's success. Darwin's naturalisation hypothesis predicts that introduced species will do better in communities where their close relatives are absent, as this will minimise niche overlap (Darwin 1859). Some studies have tested this hypothesis in microbial communities (Jiang, Tan, and Pu 2010), showing that native communities are less likely to be invaded by phylogenetically related invaders species. In the context of wine fermentation, we might hypothesise that presence of phylogenetically related species (such as other *Saccharomyces* species) may inhibit the takeover of *S. cerevisiae*. However, in other studies phylogenetic relatedness explains a minimal fraction of the variation in invasion success and so it remains unclear whether ecological invasions are predictable (Li et al. 2019). Finally, once established, *S. cerevisiae* itself can serve as a keystone species by preventing the development and invasion of spoilage microorganisms (Leale et al. 2024). For example, *Brettanomyces bruxellensis* is a common spoilage yeast that can lead to undesirable 'burnt plastic' notes in red wines. *B. bruxellensis* invasion is inhibited by *S. cerevisiae* and by the closely related *L. thermotolerans* (Leale et al. 2024). In sum, we believe that recognising ecological processes, such as a biological invasion, in the context of wine fermentation, provide invaluable theoretical basis to rationally engineering consortia that improve wine quality by promoting the growth of *S. cerevisiae* and other desirable yeast while inhibiting undesirable spoilages microbes.

5 | Evolutionary Engineering of Wine Yeast

To predict and manipulate wine fermentation, we first need to identify microbial strains whose traits contribute to desirable outcomes, either directly—through specific metabolite consumption or production—or indirectly, by influencing the growth and activity of other microbial species. Although most *S. cerevisiae* wine strains share certain traits due to a long history of domestication, significant intra-species diversity exists among these strains, which even affect central carbon metabolism (Monnin et al. 2024) and yield recognisable regional patterns in wine flavour (Knight et al. 2015). The diversity of *S. cerevisiae* strains within a single fermentation tank ranges from 1 to more than 40 (Schuller et al. 2012). Selection acting on the genetic diversity of *S. cerevisiae* strains present in grape must and winery environments drives strain dynamics, ultimately favouring the most competitive strains within the specific abiotic and biotic conditions of alcoholic fermentation. Traits such as killer toxin production, fructose preference and low nitrogen requirements are particularly advantageous in these contexts (Figure 1).

One approach for identifying strains with specific traits is to screen the existing diversity of microbes that have arisen over millions of years of evolution. For example, Pérez et al. (2021) screened 33 *Saccharomyces* yeasts for production of three different fruity ethyl esters on different nitrogen sources. Due to a common evolutionary history, closely related microbial taxa will in general tend to display more similar traits and so, having identified one strain of interest, close relatives can then be prioritised in future 'bio-prospecting' screens (Petrignani et al. 2024). In general, the success of this bio-prospecting approach depends on whether the trait of interest is phylogenetically conserved or whether it evolves rapidly and thus differs between closely related taxa. In Ruiz et al. (2023), we quantified the phylogenetic predictability of 43 wine-related traits across a library of 60 different wine yeast. We found that some traits such as ethanol production, sugar consumption or the capacity to grow in certain nutrients available in grape musts were highly predictable from phylogeny whereas other traits such as malic acid production and consumption were substantially more variable.

Once having identified a strain or set of strains leading to desirable fermentation outcomes, the next step is to try to improve on that performance. Conventionally, wine makers have done this through genetic engineering and targeted rational design either by: (i) introducing known pathways for synthesis of specific aromatic compounds into wine-adapted *S. cerevisiae* (e.g., the 'raspberry yeast' from Lee et al. 2016) or (ii) manipulating existing pathways to increase or decrease production of specific metabolites (Herrero et al. 2008; Vigentini et al. 2017). Evolutionary biology suggests significant problems with the rational design approach as engineered strains may struggle to compete with indigenous *S. cerevisiae* due to performance trade-offs (Byrne, Dumitriu, and Segrè 2012; Castle, Grierson, and Gorochowski 2021). An alternative approach to generate new strains is to rely on hybridisation which allows vast regions of genotype space to be navigated, potentially identifying new strains that can escape these trade-offs and generate fundamentally new phenotypes. For example, Bellon et al. (2013) hybridised *S. cerevisiae* with *S. mikatae* to produce stable hybrids that inherited traits from both parents and had a unique aromatic profile. Notably, in some cases, interspecific hybrids of *S. cerevisiae* and other *Saccharomyces* may be able to compete better than their parents, especially in non-standard fermentation conditions such as at lower temperature (García-Ríos and Guillamón 2022).

Directed evolution or **Adaptive Laboratory Evolution** (ALE) has also increasingly been used as a powerful evolutionary tool for engineering desirable traits into high performing strains while navigating the issues of ecological competitiveness (Walker et al. 2022; Guindal et al. 2023). In a recent study, Jouhten et al. (2022) explicitly set out to overcome performance trade-offs by evolving wine yeast strains with enhanced metabolite secretion in grape must without reducing growth rate. They first used genome-scale metabolic modelling to identify an environment in which metabolite secretion (such as production of the rose scented phenylethyl alcohol) would be coupled to growth rate. They then performed evolution experiments in these environments for 150 generations with three replicate populations. All evolved isolates maintained their growth rate on natural grape must while producing increased levels of phenylethyl alcohol. Ghiaci et al. (2024) further builds on this work by developing a new platform for massively increasing the throughput of ALE for wine-related traits. They evolved more than 9000 yeast populations simultaneously starting from different genetic backgrounds and in different environments allowing these populations to explore genotype space.

The success of these approaches and other directed evolution studies highlight the value of considering wine yeast not simply as fixed end states but also as untapped reservoirs of evolutionary potential.

6 | Conclusion

The complexity and diversity of the fermentation microbiome may appear daunting from a reductionist viewpoint: how can one possibly hope to tame a system containing dozens of different species when we can just about control *S. cerevisiae* after a century of research? We have argued that ecological and evolutionary theory can help clear the way through this complexity, providing oenologists with a solid conceptual foundation and allowing for deductive and hypothesis-driven wine research. By studying wine fermentation in its full eco-evolutionary context, oenologist can begin to understand and control the assembly of the microbial community involved in fermentation, determine the factors leading to successful *S. cerevisiae* takeover and adopt new tools for engineering more diverse array of strains and more complex multi-strain consortia. Our ultimate hope is that ecological and evolutionary theory will lead to the development of new methods for the precise engineering of multi-species microbial communities and the development of superior evolved yeasts for wine fermentation.

Author Contributions

Ignacio Belda: conceptualization, investigation, supervision, funding acquisition, resources, writing – original draft, writing – review and editing. **Javier Ruiz:** conceptualization, writing – original draft, writing – review and editing, supervision. **Belen Benitez-Dominguez:** investigation, writing – original draft. **Sergio Izquierdo-Gea:** investigation, writing – original draft. **Jean C. C. Vila:** writing – original draft, writing – review and editing, supervision.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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