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Review

Ecological principles to guide the development of crop variety mixtures

Emanuel B. Kopp[1,2], Pascal A. Niklaus[2,3,*] and Samuel E. Wuest[1,*]

[1]Agroscope, Group Breeding Research, Division Plant Breeding, Müller-Thurgau-Strasse 29, 8820 Wädenswil, Switzerland, [2]Department of Evolutionary Biology and Environmental Studies and Zurich-Basel Plant Science Center, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland, [3]University of Zurich Research Priority Program on Global Change and Biodiversity, University of Zurich, 8057 Zurich, Switzerland

*Corresponding author. E-mail: samuel.wuest@agroscope.admin.ch (S.E.W.); pascal.niklaus@ieu.uzh.ch (P.A.N.)

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Abstract

Crop variety mixtures can provide many benefits, including pathogen suppression and increased yield and yield stability. However, these benefits do not necessarily occur in all mixtures, and the benefits of diversity may be compromised by disadvantages due to increased crop heterogeneity. In-field development of mixtures by assembling many combinations of crop genotypes without prior expectation about which genotypes need to be combined to produce well-performing mixtures results in prohibitively large designs. Therefore, effective tools are required to narrow down the number of promising variety mixtures, and to then identify in experiments which of these deliver the highest benefits. Here, we first review current knowledge about the mechanisms underlying effects in ecological diversity experiments and in current agricultural applications. We then discuss some of the principal difficulties arising in the application of this knowledge to develop good variety mixtures. We also discuss non-conventional approaches to solve some of these issues. In particular, we highlight the potential and limitations of trait-based methods to determine good variety mixing partners, and argue that nontraditional traits and trait-derived metrics may be needed for the trait-based approach to deliver its full potential. Specifically, we argue that good mixing partners can be identified using modern genetic and genomic approaches. Alternatively, good mixtures may be obtained by combining varieties that respond differently to environmental variation; such varieties could easily be identified in standard variety testing trials. Preliminary analyses show that niche differences underlying the different environmental responses can indicate functional complementarity and promote mixture yield and yield stability.

Keywords agroecology, variety mixtures, crop diversity, community ecology, plant breeding, pathogen/disease control, yield stability

基于生态学原理的作物品种混合种植建立

摘要：作物品种混合种植具有很多有益效应，包括抑制病原体和增加产量和产量稳定性。然而，这些效应并不一定在所有作物品种混合种植中都出现，多样性带来的效应可能会因作物异质性的增加而抵消。在田间建立作物品种混合种植，如果没有预先预测组合哪些基因型可以产生表现良好的混合种植，会导致工作量过于庞大。因此，需要有效的工具来缩减有希望的品种混合种植组合，并在实验中确定其中哪

些组合会带来最大效应。在这里，我们首先综述已有的关于生态多样性实验和当前农业应用效应及其机理，然后讨论应用这些知识开发良好品种混合种植组合时出现的一些主要困难。我们还讨论了解决其中一些问题的非常规方法。我们特别强调基于性状确定好的品种混合种植伙伴的潜力和局限性，并认为非传统性状和性状衍生的指标可能需要基于性状的方法来充分发挥其潜力。具体而言，我们认为可以使用现代遗传和基因组方法确定良好的混合种植伙伴。或者，可以通过组合对环境变化做出不同反应的品种(这些品种可以在标准品种测试试验中轻松识别)来获得良好的混作组合。初步分析结果表明，解释对不同环境响应的生态位差异，可以指示功能互补性并提高混合种植组合的产量及其稳定性。

关键词： 农业生态学，品种混合种植，作物多样性，群落生态学，植物育种，病原物/疾病控制，产量稳定性

INTRODUCTION

Increasing crop diversity is a known path toward a more sustainable and stable crop production (Cassman and Grassini 2020; Li *et al.* 2020, 2021; Renard and Tilman 2019; Yang *et al.* 2018; Zhu *et al.* 2000). Crop diversification can be implemented at different levels, each with distinct advantages and constraints: at the very large scale, a larger diversity of crops that are grown within or between farms will contribute to food security by temporally stabilizing crop yields at the regional to global level (Renard and Tilman 2019). Within fields, intercropping of different plant species has traditionally been used to leverage benefits of beneficial interactions among crop species (mainly reduced competition, pathogen suppression and facilitation, Brooker *et al.* 2015, 2023). A somewhat similar effect can be achieved by crop rotation, i.e. by diversification in time instead of space. Finally, diversification can also be achieved within a field by co-cultivating different varieties of the same crop—which is the topic on which we focus here. Variety mixtures promise benefits similar to those seen in crop species mixtures, yet appear more compatible with modern mechanized agriculture and yield processing because they exhibit only limited heterogeneity in relevant agronomic traits (Barot *et al.* 2017; Finckh *et al.* 2000; Mundt 2002; Newton *et al.* 2009; Wolfe 1985; Wuest *et al.* 2021). Interest in the use of such variety mixtures in Western agriculture has waxed and waned over the years: the topic has a long research history (Frankel 1939; Gibson 2022), saw an interest peak in the 1980s, specifically driven by phytopathological research on diversity-mediated disease suppression, and is currently seeing renewed attention (Borg *et al.* 2018; Finckh *et al.* 2000; Wuest *et al.* 2021). In this review, we focus on variety mixtures and the current understanding of the mechanisms that underlie the benefits of such mixtures. We highlight potential bottlenecks that arise in the development of mixtures that excel on the market, in particular the problems encountered in the development and implementation of predictive methods. Finally, we re-visit ecological theory and present, based on ecological principles, a range of potential solutions to particular challenges.

FROM MONOCULTURES TO MIXTURES: KNOWN BENEFITS AND FUTURE CHALLENGES

Today, breeding programs mostly focus on developing pure lines (or hybrids) that are typically deployed in monocultures consisting of a single genotype. Breeders improve varieties by directional selection within populations that are typically created by crossing elite breeding material, which allows the accumulation of an ever-increasing number of beneficial alleles in the lines released to the market. This breeding strategy works well and has greatly contributed to increased yield and improved quality characteristics of modern varieties (Duvick *et al.* 2004; Voss-Fels *et al.* 2019). The breeding process itself may be associated with diversity bottlenecks, whereby selection against undesirable alleles results in the loss of rare but potentially beneficial alleles. This can result in a reduced representation of global genetic diversity in elite line breeding populations (Bourke *et al.* 2021; Litrico and Violle 2015; Louwaars 2018), and the overall genetic diversity within a crop may drop over time (Bonnin *et al.* 2014; Reif *et al.* 2005, but see Chai *et al.* 2022). This problem is addressed by periodically re-introducing genetic diversity into breeding populations (Gorjanc *et al.* 2016; Jannink 2010; Kovach and McCouch 2008; Reif *et al.* 2005), which over time can even increase the levels of diversity among popular varieties and improve the buffering of a crop against new challenges such as emerging diseases (Chai *et al.* 2022).

The use of pure lines grown in monoculture rests on the assumption that for a crop there is an optimal value for a trait, and that this value remains the same or at least very similar across multiple sites and years and under different management practices. However, this is not necessarily the case. It is now established that increasing within-field phenotypic variation (i.e. increasing the range of trait values) can improve across-year and across-site crop stability through a portfolio effect, and also deliver other benefits ranging from higher yield to reduced requirements for management or input (Litrico and Violle 2015). High within-field genetic variation also provides, on average, protection against disease epidemics (Box 1). However, positive mixture effects are not guaranteed (Beillouin *et al.* 2021). Indeed, some experiments have also found that genetic diversity can increase the abundance of diseases, or diversity benefits can vary strongly between experiments and even within studies (Cowger and Mundt 2002; Gibson and Nguyen 2021; Montazeaud *et al.* 2022; Mundt 2002; Smithson and Lenné 1996). In accordance, meta-analyses, while supporting the idea that mixture effects are on average positive, also show that effects are highly variable (Borg *et al.* 2018; Gibson and Nguyen 2021; Huang *et al.* 2012; Kiær *et al.* 2009; Koricheva and Hayes 2018; Kristoffersen *et al.* 2020b; Reiss and Drinkwater 2018; Smithson and Lenné 1996). Specifically, they show that (i) overyielding benefits average around 2%–4% (slightly higher effects are found under low-input conditions); (ii) diseases are, on average, suppressed in mixtures, but effect estimates are difficult to extrapolate from experiments to real-world applications, because many epidemiological phenomena only appear at scales larger than the ones used in experiments (Box 1) and (iii) mixture yield stability is often slightly improved compared with monocultures. Increased yield and stability are, however, often more pronounced in mixtures under more variable conditions or disease pressure.

To summarize, the transition from traditional cropping systems that utilized genetically diverse populations (landraces) to monocultures of pure lines decreased within-field heterogeneity (Dawson and Goldringer 2011; Harlan 1975). While management and production were simplified in this process, the potential advantages that diversity provides were lost. This may become particularly critical when facing future environmental challenges such as climate extremes and the emergence of novel pathogens (Bonnin *et al.* 2014). Indeed, breeders are concerned about the increasing difficulty to identify new genotypes that perform well across the whole range of their target environments (Dario Fossati, personal communication).

CHALLENGES IN BREEDING FOR VARIETY MIXTURES

Overall, existing research demonstrates that mixtures can provide tangible benefits, especially when good variety compositions can be identified. Which compositions are best depends on goals and applications, but in general the properties aimed for will be high yield and yield stability, a good suppression of pathogens, and a high quality of the product. However, developing mixtures adds another level of complexity to today's crop development programs. Breeders are already facing growing lists of breeding aims to address new problems such as increasing climatic uncertainty or the unprecedented spread of new pathogens (Fones *et al.* 2020; Xiong *et al.* 2021). Breeders are therefore wary of additional constraints such as the requirement to minimize genetic variation for traits for which uniformity is desirable (e.g. agronomic traits such as phenology including maturity date) but to maximize genetic variation for the traits that underpin the diversity benefits (Litrico and Violle 2015; Wuest *et al.* 2021). At the same time, concerns about conflicts of phenotypic diversity with market demands for uniform products are often unfounded (Mundt 2002; Newton *et al.* 2009, D. Fossati personal communication). Mixture developers, however, focus more strongly on negative selection criteria (e.g. agronomic constraints that demand the exclusion of specific variety combinations like large maturity date differences, very different quality traits, etc.), further increasing the disincentive to develop variety mixtures at all. A general mechanistic understanding of diversity effects would likely change this: e.g. a recent meta-analysis of wheat variety mixture experiments showed relatively higher overyielding values reported in studies from the 1980ies than in more recent ones, likely due to a strong focus on disease suppression mechanisms during that time period and leading the development of more disease resistant—and more productive—mixtures (Borg *et al.* 2018).

Another challenge lies in the combinatorial properties of mixtures: designs to combine pure lines

BOX 1: PATHOGEN SUPPRESSION—THE ULTIMATE ECOSYSTEM SERVICE?

The use of genetic diversity to mitigate large-scale disease epidemics and to suppress local pathogen spread has been of interest for more than a century (Gibson 2022), and mixture-mediated reductions in pesticides can likely make a major contribution toward sustainable agriculture (Mundt 2002; Zhu *et al*. 2000). Much of the phytopathological work so far has focused on the question whether multilines, mixtures or heterogeneous populations suppress pathogens and pests better than do monocultures, and under what conditions (e.g. ratio of resistant to susceptible plants, planting densities, level of disease pressure or type of pathogen). Numerous studies now summarized in several meta-analyses and reviews have established that, on average, mixtures are effective at suppressing diseases, and increase yield under high pathogen pressure (Cowger and Mundt 2002; Finckh *et al*. 2000; Finckh and Wolfe 2015; Kiær *et al*. 2009, 2012; Kristoffersen *et al*. 2020b; McDonald *et al*. 1988; Mundt 2002; Reiss and Drinkwater 2018; Smithson and Lenné 1996; Yang *et al*. 2019; Zhu *et al*. 2000). However, many questions still remain to be answered before a richer understanding or effective predictive methods for disease suppression can be reached. So far, a number of potential mechanisms for disease suppression in mixtures have been proposed (Borg *et al*. 2018; Finckh *et al*. 2000; Mundt 2002), e.g. epidemiological effects through a reduced density of susceptible hosts, or even non-hosts that act as barriers for the spread of an inoculum. Further, an infection of a host with a pathogen strain i.e. incompatible may trigger increased host immunity, and therefore represent an early-warning signal and increased resistance against compatible pathogen strains. Competitive interactions may also increase basal pathogen resistance, through mechanisms are not fully understood (Barton and Bowers 2006; Pélissier *et al*. 2021). Finally, resistant hosts may compensate for the losses suffered by susceptible neighbor plants, leading to higher fractions of resistant host tissue within a field (Finckh 1992). It is worth noting that disease-suppressive effects not only arise in mixtures that were deliberately designed to do so (e.g. based on combining varieties with different resistance genes), but also in cases they were not (Kellerhals *et al*. 2003; Kristoffersen *et al*. 2020b), suggesting that plant–pathogen interactions are more complex than early models have implied (Flor 1971; Wu *et al*. 2018) A still understudied potential of mixtures is therefore their ability to simultaneously suppress multiple pathogens (Finckh *et al*. 2000; Wuest *et al*. 2021), e.g. when each component of a mixture shows resistance to a different pathogen. Such mixture properties could be specifically used in breeding programs, and future research may extend the focus on the general nature of host–pathogen network structures (i.e. interactions of varieties and pathogens or pathogen strains) that allow for particularly effective disease suppression in mixtures.

Provided many studies that have shown a disease-suppressive effect of mixtures compared to the corresponding monocultures, the research focus should now shift toward the underlying mechanisms, using methods that compare different mixtures, ideally in large numbers, to determine or confirm predictions when diversity effects differ in size. Diallel or factorial designs, typically used in hybrid breeding, are ideally suited for this purpose (see main text and Fig. 3). However, some epidemiological control mechanisms, such as reduced host density or barrier effects are typically dependent on the scale at which mixtures are grown and evaluated, and on the mode of pathogen dispersal (Finckh *et al*. 2000; Mundt 2002): diversity effects on long-distance wind-dispersed wheat mildew or rusts are hard to score in small experimental evaluation plots due to large effects of external inoculum pressures. Such scale dependencies are *per se* an important research topic that will likely benefit from support by epidemiological models (Garrett and Mundt 1999; Mikaberidze *et al*. 2015). Finally, repeated breakdowns of resistances upon the evolution of new virulent pathogen strains, also referred to as 'boom-and-bust cycles' in plant pathology (Brown 2015; McDonald and Linde 2002; Mundt 2014; Wolfe and Barrett 1980), can have devastating consequences for crop production and diminish the pool of resistance genes available for the breeder. Combining a variety of resistances at different spatial or temporal scales has been proposed as a solution to this problem (Brown 2015; Finckh *et al*. 2000; McDonald and Linde 2002; Mundt 2014; Rimbaud *et al*. 2018, 2021). Mixtures may contribute to such resistance management strategies in the future, but investigating processes at such large temporal and spatial scales using experiments represents an enormous challenge (Finckh *et al*. 2000; Garrett and Mundt 1999; Kristoffersen *et al*. 2020a; Rimbaud *et al*. 2021).

into different variety mixtures quickly escalate because in the case of two-component mixtures the number of possible combinations increases with the square of the number of components, and even more rapidly for more complex mixtures (Barot *et al*. 2017; Wuest *et al*. 2021). This makes it impractical to test even a fraction of all possible compositions. More efficient designs can alleviate this problem (see below), but finding variety combinations that result in a mixture benefit remains difficult and is often perceived as requiring larger efforts for field testing than can be justified based on the expected potential returns.

For these reasons, the full potential of mixtures likely remains untapped, although mixtures promise to reduce pathogen epidemics and improve the crop's resource-use efficiency, goals that rank high on today's agendas. One key innovation to promote mixtures would therefore be to identify effective methods to design high-performing mixtures whose benefits outweigh the disadvantages. In the following, we therefore discuss the following questions: (i) what insights have been gained in ecological studies of species diversity effects and in intercropping, and (ii) how could such insights suggest positive selection criteria for variety mixture development. We also identify specific applications of ecological principles that have not received much attention but could be of great use in practical mixture designs, especially when combined with current agronomic and breeding methods.

ECOLOGICAL THEORY RELATED TO BIODIVERSITY BENEFITS

Positive species diversity effects on grassland productivity are well documented: a large body of observational and experimental studies have shown that more diverse plant communities often are more productive and their productivity temporally more stable than less diverse plant communities (Cardinale *et al*. 2011; Isbell *et al*. 2015; Liu *et al*. 2021; Morin *et al*. 2014; Proulx *et al*. 2010). In general, the diversity–productivity relationship is 'positive decelerating', i.e. adding additional species results in decreasing marginal increases in productivity as community diversity increases. There is broad consensus that biodiversity effects can be attributed to some form of functional complementary among species. This functional complementarity has been described in terms of niche differences among species, reasoning that distinct species compete less because they 'partition niches', and, together, constitute a broader 'community niche' (Salles *et al*. 2009; Turnbull *et al*. 2016). However, the exact mechanisms that underlie such complementarity have proven very difficult to identify. An early idea was that abiotic resources such as soil nutrients are partitioned, but to date there is little direct empirical evidence for this (McKane *et al*. 2002; Silvertown *et al*. 1999; Von Felten *et al*. 2012), despite decades of research, except for simple and relatively obvious cases (e.g. symbiotic nitrogen fixation in legumes, see Spehn *et al*. 2002). Another idea is that plant community members 'partition' interactions with enemies such as herbivores and pathogens, i.e. enemies are specialized to different hosts. There is evidence that this supports biodiversity effects (Huang *et al*. 2022; Maron *et al*. 2011; Mikaberidze *et al*. 2015; Schnitzer *et al*. 2011), but also here many aspects remain unclear. Species may further facilitate the growth of other species, e.g. by environmental niche construction (Wright *et al*. 2017). Overall, subsuming all these (and likely also further) mechanisms in the concept of ecological niches results in a niche concept i.e. highly abstract (Chase and Leibold 2003). It is useful in mathematical models because many phenomena can be parameterized using the same form (e.g. coefficients characterizing species interactions). However, the concrete niches and niche dimensions remain difficult to quantify in practice.

Because niches are so difficult to quantify directly, an appealing alternative is to infer niches indirectly from plant traits (Box 2). The underlying reasoning is that niche differences must be related to phenotypic differences that reflect the capabilities of a species to 'deal' with the challenges posed by its abiotic and biotic environment. While this certainly is true, a number of difficulties arise. First, the traits considered may not be aligned with the fundamental niche dimension of interest (Blonder 2018; D'Andrea and Ostling 2016; Funk and Wolf 2016; Kunstler *et al*. 2012, 2016). For example, a shift along a specific niche dimension that underpins complementarity may result in a whole suite of trait changes, and these trait changes may be nonlinearly related to the original shift along the niche axis (Hoogenboom and Connolly 2009; Porter and Kearney 2009). Second, traits typically form syndromes of correlated changes because they together mediate an environmental adaptation (Díaz *et al*. 2015; Wright *et al*. 2004), or because trade-offs exist in terms of the underlying physiology, morphology or genetics. Third, not all trait differences may be functionally important, at least not in the studied context. And finally, there may be traits that are difficult to observe because they are related to specific physiological functions that are not reflected in morphological differences. One example may be different root uptake kinetics for nutrients. Probably for all these reasons, trait-based approaches have not lived up to the expectations with which they were originally adopted in experimental biodiversity research. Today, there is consensus that single traits only have limited predictive power (van der Plas *et al.* 2020), and that niche complementarity likely is higher dimensional in terms of the associated trait space. Also, it may be that the specific traits mediating complementarity differ between different species compositions of mixtures, e.g. between different species pairs considered (Kraft *et al.* 2015).

ECOLOGICAL PRINCIPLES APPLIED TO AGRICULTURE

How do these ecological concepts perform in agricultural applications? Interestingly, for species mixtures (intercropping), there are many cases in which the mechanisms responsible for the community-level benefits are relatively well understood (Brooker *et al.* 2015; Homulle *et al.* 2022; Schöb *et al.* 2023). An obvious case is the use of legumes, which can meet their nitrogen demand from atmospheric N_2, thereby avoiding competition for soil nitrogen with other species. In the long term, N_2 fixation results in a general enrichment of the ecosystem with nitrogen and hence higher productivity also in non-fixers (Annicchiarico *et al.* 2019; Cowden *et al.* 2020; Engbersen *et al.* 2021). Many further examples exist: in agroforestry systems, trees provide shade to crops and increase water availability to the less deep-rooting crops by hydraulic lift—a case of facilitation by environmental niche construction (Alagele *et al.* 2021; Brooker *et al.* 2015; Homulle *et al.* 2022). Peas often are co-cultivated with cereal crops (Hauggaard-Nielsen *et al.* 2001). Here, the cereal prevents pea lodging and suppresses weeds by improving ground cover—mechanisms that could be termed niche construction and indirect biotic facilitation. In maize–faba bean intercrops, beans were found to mobilize phosphorus through local modifications of soil properties, thereby improving growth conditions also for maize (Li *et al.* 2007). Because in these (and similar cases) the main mechanism of interaction is relatively well understood, such systems are amenable to improvement by 'engineering approaches'.

At the intraspecific level, in general, genotypes of a species are more similar than different species. Nevertheless, positive effects of genotypic diversity have not only been documented in agricultural variety mixtures but also in ecological studies (Bukowski and Petermann 2014; Cook-Patton *et al.* 2011; Crawford and Whitney 2010; Schmid 1994). The challenge in identifying the mechanisms underlying these intraspecific diversity effects is that here the mixture components do not combine very dissimilar functions (e.g. a N_2-fixer with a non-fixer, or a plant providing mechanical support to another). Instead, complementarity is rooted in traits that often are less visible to the human observer, because the varieties' morphologies are relatively uniform.

BOX 2: TRAIT-BASED METHODS IN ECOLOGY

Trait-based methods allow to establish general rules applicable to a broad range of ecological patterns and processes, e.g. those underlying the assembly and functioning of plant communities (Grime and Pierce 2012; Shipley *et al.* 2016; Violle *et al.* 2007). A prominent example is the leaf economic spectrum (LES) that describes a continuum of plant growth strategies i.e. readily described by six functional leaf traits related to fundamental resource allocation trade-offs (Wright *et al.* 2004). This relationship holds across a wide range of habitats, despite the vast diversity of species and their often idiosyncratic properties. Many readily observable plant traits also reflect adaptations to specific environments and are therefore interpreted as proxy for a species' niche position along specific dimensions of the Hutchinsonian niche (Fig. 1). These traits successfully predict species abundances and habitat filtering processes when moving along gradients in e.g. precipitation, altitude or latitude (Cadotte *et al.* 2015; Chalmandrier *et al.* 2017; Schellenberger Costa *et al.* 2017). Unsurprisingly, trait-based methods have become a central part of functional ecology (Shipley *et al.* 2016; Violle *et al.* 2007). However, the application of trait-based concepts to predicting the performance of mixtures of species (or varieties) has turned out to be surprisingly challenging. In general, traits seem to predict only a relatively small fraction of the observed variation in ecosystem functioning

(Ebeling *et al*. 2014; Roscher *et al*. 2012; van der Plas *et al*. 2020). A reason may be that the traits used in these studies do not reflect the complementarity of co-existing species adapted to the same set of broad environmental conditions, and that the particular traits mediating complementarity are not well understood to date (Fig. 1).

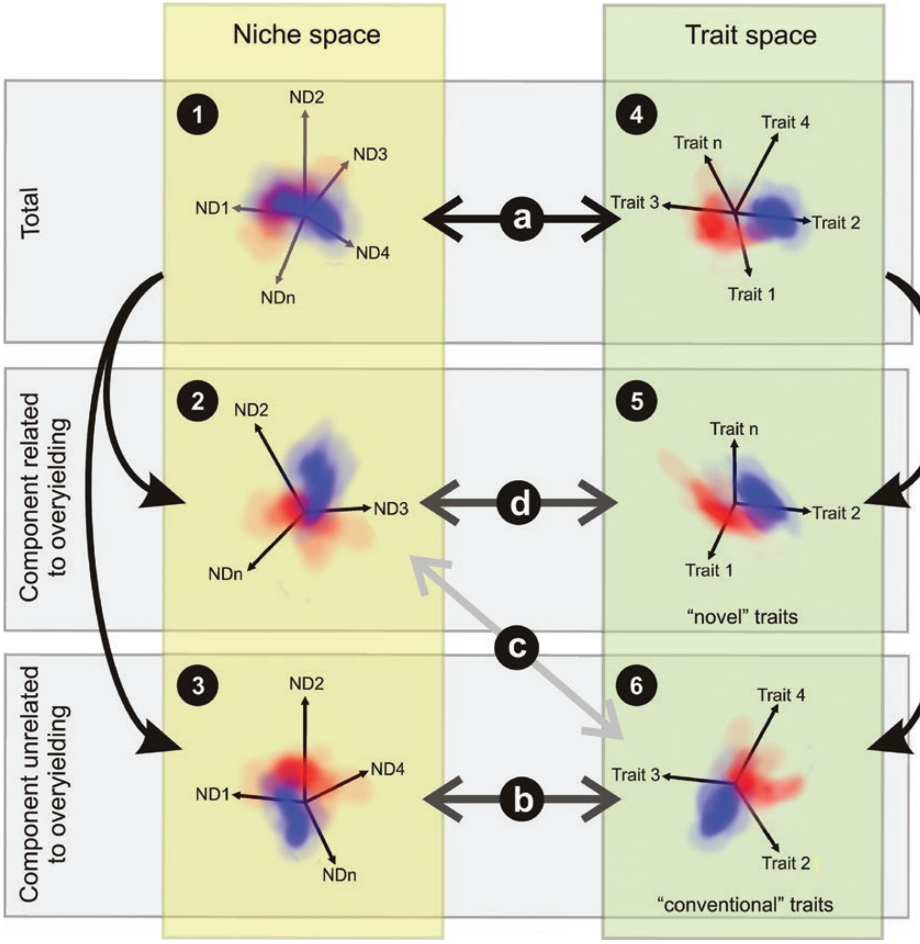

Figure 1: Niches and traits. Niches are often defined in a high-dimensional space spanned by axes (ND1, ND2, …, ND*n*) that correspond to a species' environmental requirements (e.g. climate, edaphic conditions, soil nutrients; Hutchinson 1978) and interactions with other organisms (e.g. competitors, consumers, symbionts, pathogens; Chase and Leibold 2003). The space (hypervolume) that contains the conditions under which the species persists then defines its niche (1, red and blue volumes indicating the niches of two hypothetical species. Note that the sketch is conceptual because niche overlap cannot be adequately shown in two dimensions). Because the niche of a species is related to its interactions with its biotic and abiotic environment, which in turn depend on the species' functional characteristics, it is evident that niches have a correspondence (a) in trait space (4). In functional ecology, niche dimensions therefore often are approximated by sets of observable traits. When applying this idea to the analysis of biodiversity effects, difficulties arise: diversity effects evidently root in some form of niche partitioning; however, it is equally evident that not all niche differences promote diversity effects. Hence, only a component of the total interspecific niche differences underlies diversity effects (2); the remaining niche differences (3) are unrelated to diversity effects but matter in other contexts. Here, we posit that the suite of traits commonly used in ecological research (e.g. SLA, LDMC, leaf N contents) (6) strongly correlate (b) with niche dimensions related to fundamental growth strategies (e.g. leaf economic spectrum) and environmental adaptations, but are not or only weakly related to niche dimensions underlying diversity effects (c). 'Novel' trait metrics (5) that capture less-obvious characteristics of a species, and that are less related to environmental adaptations may better predict species differences that promote diversity effects (d). An example of such traits are pathogen susceptibilities and defense mechanisms, characteristics that are known to matter for enemy-related diversity effects (Box 1) but are not included in the 'conventional trait set'.

A prominent example of diversity effects in variety mixtures are differences in the susceptibility of varieties to particular pathogens or pathogen strains. By 'partitioning' the host–pathogen interactions, varieties will benefit from lower host densities available to specialized pathogens. Because the dynamics of epidemics are strongly density dependent, a general suppression of diseases and associated yield losses often is observed (Kristoffersen *et al.* 2020b, 2022; Reiss and Drinkwater 2018). However, mechanisms beyond simple density dependence also contribute much to disease suppression (Finckh *et al.* 2000; Mundt 2002 and Box 1).

UNTAPPED POTENTIAL FOR COMPLEMENTARITY

In both ecological experiments and in agriculture, overyielding is frequently observed—yet, it seems rather the exception (e.g. Finn *et al.* 2013; Gibson 2022) than the rule that the specific mechanisms responsible for these effects are understood in detail (Barry *et al.* 2019; van der Plas *et al.* 2020), in particular in variety mixtures in which the observable differences between mixture components are more subtle (Montazeaud *et al.* 2018, 2020).

What are the reasons for this difficulty to identify traits that cause overyielding? First, ecological research related to traits has largely focused on niche dimensions that are related to adaptations to environmental conditions or characteristic of a relatively narrow set of ecological strategies. Hence, these traits mainly describe the suitability of a species to a particular environment, e.g. adaptation to aridity, or whether it is characterized by rapid, acquisitive or slow, conservative growth (Díaz *et al.* 2015; Wright *et al.* 2004). While these are important traits, we argue here that other traits that receive less attention likely are more important mediators of complementarity *within* a given environment (cf. Box 2). Second, complementarity likely is related to a (possibly nonlinear) combination of a larger trait suite, especially when the traits considered are not the ones closely linked to the functional differences that ultimately cause complementarity (Chacón-Labella *et al.* 2022). Third, within a given environment, the relevant trait differences may be relatively subtle and plastic, so that complementarity only becomes apparent in visible trait differences once the respective components interact. Based on all these considerations, we therefore posit that a large fraction of existing complementarity currently remains hidden because it remains inaccessible using the traditional trait-based methods. It thus seems that alternative approaches are required to uncover this potentially large 'complementarity reservoir'.

NOVEL IDEAS TO TACKLE AN OLD PROBLEM

At the root of the problem discussed so far is the lack of clear hypotheses regarding the concrete biological mechanisms that underlie complementarity—therefore, the selection of traits is rather opportunity-driven than hypothesis-driven (i.e. the focus is on traits that are already well described and easy to measure). Given that plant interactions are complex and not particularly well understood, this is unlikely to change in the near future. In the end, this means that agnostic methods are required that broaden the trait domain investigated to date.

One idea is that genotype-by-environment interactions could be used to this end (Fig. 2). In classical ecological work, niche overlap is frequently determined by counting the occurrence of individuals of different species in different microhabitats, or their use of different food sources (Colwell and Futuyma 1971; MacArthur 1958; Pianka 1973, 1974). Species that co-occur frequently across microhabitats, or that share the same food sources, are considered to occupy similar niches. In analogy, proxies of niche overlap between crop varieties could be derived from pure-line yield changes across multi-year, multi-site variety trials, which are commonly performed during breeding and variety testing The underlying rationale of using this data to identify complementary varieties is that year × site combinations differ in many aspects (e.g. edaphic and climatic conditions, disease pressure), some of which remain unknown, and that the 'specialization' of varieties is expressed when they are confronted with such different environments. Similarly, genotype-by-environment interactions in disease susceptibility, which can be considerable in field trials (Beukert *et al.* 2020), could be a sign that genotypes are specialized in their 'pathogen niches' and resistant against different strains (which occur at different frequencies at different sites or years), or that some unknown environmental factors differentially modulate resistance traits in different genotypes. In a recent proof-of-concept analysis we have shown that overyielding of variety mixtures in Danish wheat trials can indeed be predicted from variety-specific pure stand yield variation across years and sites (Wuest *et al.* 2021). This suggests that the

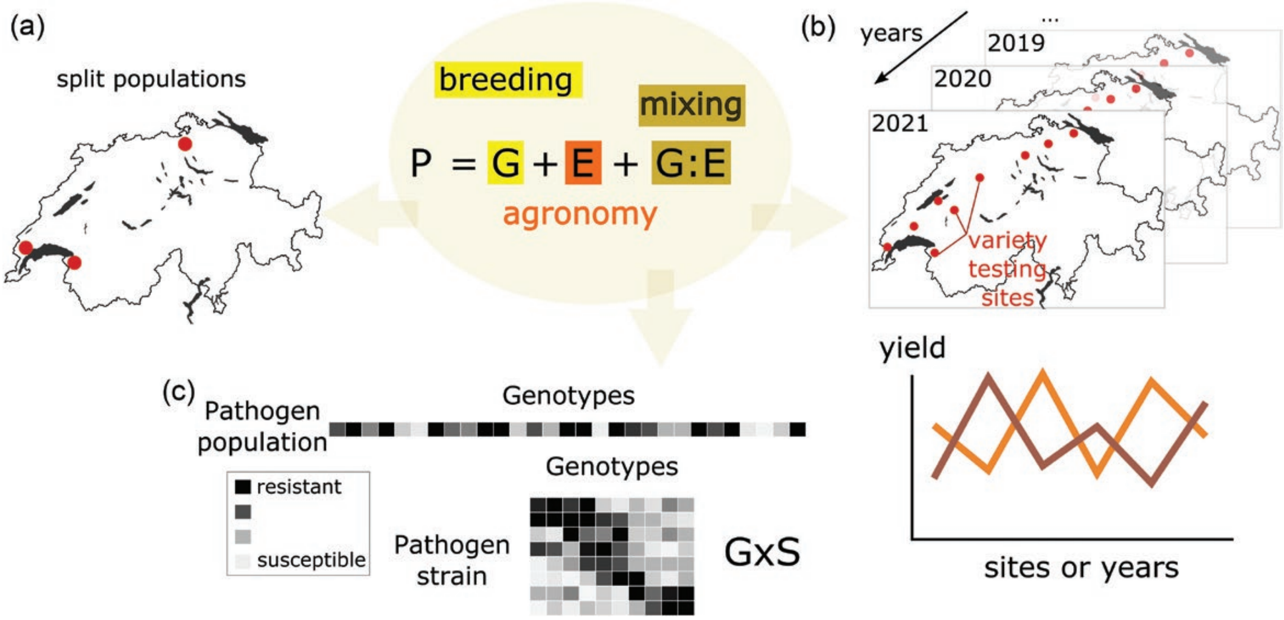

Figure 2: Ecological specialization of varieties through diverse strategies. Breeder's partitioning of phenotypic variation (P) into various components highlights different levers for crop improvement. G represents a genotypic variance component, the additive part of which influences the response to selection that can be achieved through breeding. E represents an environmental variance component (e.g. variation between sites, year or even management), which is also influenced by agronomic practices such as fertilization or irrigation. The G × E component is typically considered a nuisance for breeders but could be exploited to some degree in mixture development. Three examples are given here, as follows: (**a**) Splitting a breeding population across sites with different pedoclimatic conditions or pathogen pressures, etc., representing relevant niche axes along which genotypes could be specialized for optimal complementarity. (**b**) Asynchrony of genotypes across variety testing sites or years in multi-environment trials typically conducted in breeding and variety testing may stabilize mixture yields and even lead to overyielding or other mixture benefits, as described in Wuest *et al.* (2021). (**c**) Genetic resistances are typically evaluated upon infecting different genotypes with a pathogen population or a single pathogen strain. However, modeling work shows that genotypic specialization for different pathogen strains (i.e. how specific genotypes interact with specific pathogen strains—genotype-by-strain (G × S) interactions), or alternatively, genotype-by-environment interactions in disease susceptibility, should result in maximal mixture benefits and could also slow the evolution of new virulent strains.

specialization of varieties, and the resulting 'division of labor' that supports overyielding, indeed is reflected in differences in *environmental reaction norms*, i.e. as differences in variety-specific trait changes across environments (Fig. 2b). In the case of variety multi-environment trials, such reaction norms likely represent multiple environmental dimensions, which is maybe better aligned with multivariate theories of plant species coexistence (Kraft *et al.* 2015). This approach allows for the estimation of variety niches based on data readily available from standard variety development trials, by calculating differences in environmental reaction norms between genotypes instead of focusing on single trait differences between varieties within environments. Another advantage of this approach is that differences in the function of varieties are assessed in the environmental range for which the cultivar is suitable. This somehow contrasts with approaches that focus on determining the environmental range of a species—here the focus is rather on the margins of the niches.

This idea can not only be applied to mixture productivity (by increasing overyielding) but also to tackle the stability of mixture yields. A tenet in community ecology is that the greater stability exhibited by more diverse communities is driven by higher asynchrony among component species populations. Again, we argue that such asynchrony can be measured in analogy in pure stand plots assessed across different sites and years. A preliminary test we performed using the same Danish trial dataset indicated that this approach indeed predicted a significant amount of variation in mixture stability (Wuest *et al.* 2021). The idea to leverage such differences runs counter prevailing breeding practice. The phenotypic variation observed in breeding trials can be partitioned into genotypic variance, environmental variance and variance due to genotype-by-environment (G × E) interactions. Breeders generally concentrate on the genotypic variance component, in particular its additive part, on which selection can operate and which drives breeding progress. G × E interactions *within the defined production environment* targeted by the breeders are seen as a nuisance because further niche breeding is not economical. However, niche differences and asynchrony as evidenced in growth differences of pure stands across different environments are drivers of such G × E interactions. In other words, mixture development could build on a component of phenotypic variance i.e. inaccessible and generally undesired in pure-line breeding. This offers the potential for important synergies between breeding and mixture development.

A fundamental alternative to focusing on traits as predictors of complementarity is to use genetic methods to identify the genetic underpinnings of community overyielding (Montazeaud *et al.* 2022; Sato *et al.* 2021; Wuest and Niklaus 2018; Wuest *et al.* 2023). In some pilot studies with mixtures of model plants, we have been able to link overyielding to genetic diversity across genotypes at particular loci. Such insights may be directly applicable in breeding. In the longer term, understanding the genetic basis of diversity effects may also lead to the identification of associated functional traits. In our studies, some diversity effects were mediated indirectly through soil factors (e.g. Wuest and Niklaus 2018; Wuest *et al.* 2023), and associated with root physiology. These functional differences were not apparent in the widely adopted 'conventional' traits such as leaf angle, plant height or specific leaf area, and if they had been apparent, then these differences likely would have been a consequence of modified growth rather than its primary cause.

Once, in one way or another, causal mechanisms or specific trait combinations leading to complementarity have been identified, it will be relatively straightforward to select complementary varieties that optimize yield, yield stability, suppress diseases or promote other ecosystem functions (Barot *et al.* 2017). With the leading niche dimensions identified along which varieties can be specialized for optimal complementarity (e.g. specific nutrients, soil properties, pathogen communities), new selection strategies could be implemented in breeding programs. For example, breeding populations could be split among field sites that span the relevant niche axis, and divergent adaptation to local sites and conditions should then select for complementarity among these split populations (Fig. 2a). Such niche breeding would also account for genotype-by-environment interactions that are normally deemed unfavorably by breeders, and which are often caused by trade-offs between alternative plant strategies. Directional selection in split populations will follow already established breeding methods but lead to local adaptations that can lead to within-mixture complementarity. Furthermore, populations can be genetically differentiated in analogy to genetic differentiation i.e. used in hybrid breeding schemes, where heterotic groups are bred separately before hybrid crosses. Modern genomic technologies could make such differentiation highly efficient (Li *et al.* 2022; Technow *et al.* 2014; Zhao *et al.* 2015).

Nevertheless, the combinatorial challenges associated with mixture testing remain: optimizing mixtures, regardless of the procedure, requires the screening of a large candidate pool of mixtures. In ecological diversity experiments, overyielding is assessed relative to the performance of monocultures of the components. For variety mixture development, this is inefficient because monocultures are not the communities of interest, yet monocultures need to be highly replicated to provide statistically stable benchmark values to assess overyielding. Also, in mixture development the goal has become to identify the best mixture in a set of mixtures, and no longer to quantify performance of these mixtures relative to monocultures not intended for cultivation. Both problems can be circumvented using diallel designs (Fig. 3) in which the average performance of a mixture is assessed relative to the average performance of the components across all tested mixtures, and potentially even in a range of environments (Forst *et al.* 2019; Griffing 1956; Norrington-Davies 1967; Schmid *et al.* 2002; Wuest and Niklaus 2018). Such designs represent very effective starting points to determine additive contributions of varieties to mixtures, and to evaluate how varietal differences improve their interactions and thus the performance of specific mixture compositions. Such designs, and variations thereof (Forst *et al.* 2019), therefore shift the focus from comparisons between monocultures and mixtures toward the identification of characteristics that make some variety mixtures more effective than others. Whether these characteristics are best described by differences in genes, in biochemical properties, in physiological or morphological traits, in reaction norms, or in some other metrics is currently unclear. However, identifying such predictors is highly relevant for both mixture development and

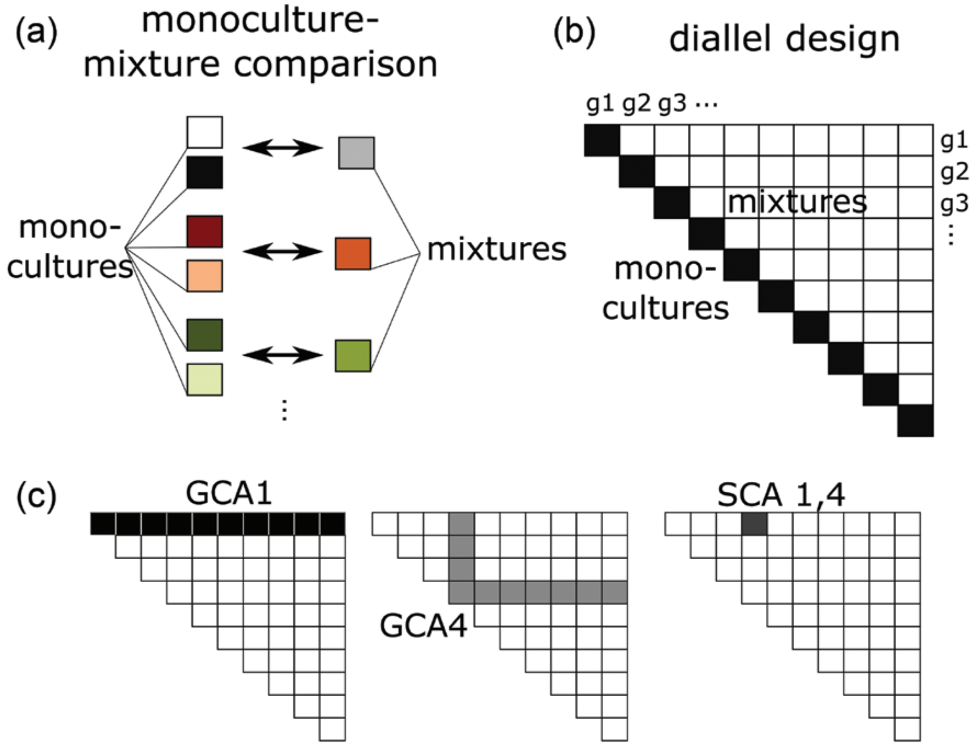

Figure 3: Shift from a focus on monoculture–mixture comparisons (**a**) toward systematic comparisons between different mixtures will require alternative experimental designs. Diallel designs (**b**) systematically pair different combinations of genotypes or varieties (g1, g2, g3, …) and focus on comparisons among mixtures. (**c**) Additive performance contributions (biomass, yield, ….) of genotypes across all mixtures (i.e. how much a genotype on average contributes to the mixture performance, termed general combining abilities—GCA; examples shown are GCA1 and 4, which are estimated from the average performance of the shaded compositions) can be modeled from diallel designs without the need to grow monocultures, and allow the formulation of expected mixture performances based on additive contributions only. Expected deviations of genotype combinations from such additive expectations are termed specific combining abilities (SCA) and can be utilized to understand how trait, gene or other differences between genotypes contribute to complementarity and the added value of specific mixtures.

for ecologists studying biodiversity effects and species coexistence.

CONCLUSIONS

Increased diversification, ideally at different spatial or temporal scales, represents a known strategic element of a sustainable agricultural intensification. In isolation, variety mixtures are unlikely a 'silver bullet' to overcome all major challenges in mechanized agriculture. At the same time, their widespread use could make important contributions to solving some important problems or current trade-offs, and could contribute at multiple levels to improved crop functioning and reduced input. However, transitioning from pure-line breeding to the widespread use of variety mixtures is associated with different challenges. The ways in which such challenges can be overcome will determine if this transition is deemed economical by the wider breeding and seed-production community, and if high-performing mixtures—with multiple benefits—can be developed that are then embraced by farmers and the processing industry. As we outlined here, ecological principles can guide efforts to address many of these challenges. We have highlighted that ecological theory and the knowledge gained from the study of biodiversity–ecosystem function relationships can be combined with the resources and data obtained by default from breeding or variety testing activities, generating synergies that could tip the perceived economic imbalance between pure cultures and mixtures. Whereas trait-based approaches to predict mixture performances are conceptually attractive and have shown their merits in specific situations, they should not distract from a larger pool of alternative strategies, also rooted in ecological theory, to be explored in the future, namely the utilization of reaction norms to approximate variety niches, mechanism-agnostic genetic approaches and diallel designs which shift the focus from mixture–monoculture comparisons to mixture–mixture comparisons. Time will tell if the more empirical, 'engineering' approaches or the more theory-driven methods discussed here will provide better guidance for mixture development, but both will likely enable a better use and understanding of the positive effects of diversity in agro-ecosystems.

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