Letters

Evolutionary bet-hedging in arbuscular mycorrhiza-associating angiosperms

Early terrestrial plants colonizing land probably relied on arbuscular mycorrhizal (AM) associations to meet their nutrient needs (Smith & Read, 2008; but see Bidartondo *et al.*, 2011). Despite occasional diversifications towards other mycorrhizal association strategies (Hoeksema, 2010; Feijen *et al.*, 2018), the AM symbiosis shows a remarkable persistence over evolutionary time (Brundrett & Tedersoo, 2018). Not all plants, however, benefit equally from associating with mycorrhiza (Wilson & Hartnett, 1998), and it remains unclear why some plant species for which we often observe negative responses (such as *Bromus inermis*, *Poa pratensis* and *Koeleria pyramidata* in Wilson & Hartnett, 1998) to mycorrhiza continue to associate with AM fungi. An obvious shortcoming of many experimental studies using arbuscular mycorrhiza is that for logistical reasons they are carried out over relatively short periods. In the long run, the fitness of an organism is an expression of the geometric, and not the arithmetic, mean of payoffs realized over generations (Sæther & Engen, 2015), which means that expectations from short-term studies might be biased and overstate benefits (or lacks of benefits) from AM fungi (Supporting Information Fig. S1a; Notes S1). Here, we explore the possibility that, over evolutionary time, AM fungi benefit plant hosts by enabling them to survive unfavourable events at the expense of a relatively lower fitness (compared to a noncolonized state) under less stressful periods, in what is known as 'evolutionary bet-hedging'.

A well-described case of evolutionary bet-hedging across biological systems is the evolution of dormancy in plant seeds (e.g. Evans, 2005; Childs *et al.*, 2010): natural selection has resulted in the ability of seeds to germinate over several years, potentially to account for environmental stochasticity. There have been, however, observations of evolutionary bet-hedging across many organisms spanning the tree of life, such as viruses (to the degree that they can be classified as living organisms; Maslov & Sneppen, 2015), bacteria (Beaumont *et al.*, 2009), fungi (Levy *et al.*, 2012) and vertebrates (McAllan *et al.*, 2012). Lekberg & Koide (2014) proposed that AM-associating plants engage in associations with less beneficial AM fungi as part of a bet-hedging strategy and possibly AM fungi do the same in relation to partner choices (Babikova *et al.*, 2013; Veresoglou & Rillig, 2014). Field *et al.* (2015) formulated a very similar hypothesis in relation to symbiotic partner choices in early terrestrial plants. Bet-hedging (but also evolutionary bet-hedging) in AM associations could also arise, however, if upon colonization plants can better tolerate transient environmental stress such as droughts (e.g. Augé, 2001). To the best of our knowledge, the possibility of evolutionary bet-hedging by plants forming symbioses with AM fungi has not been addressed.

We propose three different scenarios that could give rise to an evolutionary bet-hedging strategy in AM-associating plants (Fig. 1). The most intuitive scenario (*Scenario A*) describes strong positive mycorrhizal responses during adverse years which offset likely negative growth benefits over favourable years (Fig. 1). The scenario shares expectations with nonevolutionary forms of bet-hedging (describing bet-hedging that occurs at timescales of a single generation such as how mycorrhiza can promote plant fitness under adverse soil conditions but simultaneously suppress it in areas of high fertility; Lekberg & Koide, 2014) and thus shares possible mechanisms with them. One possible mechanism is better protection from pathogens (Veresoglou & Rillig, 2012), which could slow down adaptations to low phosphorus (P) availability, giving rise to a tradeoff between pathogen protection with mycorrhiza and low-P tolerance without mycorrhiza (Laliberté *et al.*, 2015). A further mechanism is improved tolerance to extreme weather conditions (e.g. Auge, 2001). Here, an evolutionary bet-hedging strategy could also arise if AM fungi reduce relative fitness differences across hosts (as shown in Veresoglou *et al.*, 2018) under different weather conditions. This may lead to plants that are disadvantaged by weather conditions exhibiting reduced fitness losses relative to plants favoured by the weather conditions.

In *Scenario B*, climatic conditions do not necessarily modify average growth effects of mycorrhiza, but AM fungi stabilize plant fitness in time (i.e. reduce temporal variability, which is often assayed in experimental studies as the coefficient of variation of a metric of fitness in time). In the longer term (i.e. when we encounter over 10 generations), fitness depends on the geometric mean (and not the arithmetic mean; Notes S1) of growth effects in time, implying that organisms that on average experience a reduction in fitness gains, can still have greater fitness if they experience lower temporal variability (Fig. S1; see Methods S1 for a reproducible example). There have been several recent studies addressing how mycorrhiza alters the temporal variability of plant fitness (in most cases in the form of biomass production) and most studies report that AM fungi reduce temporal variability (and thus support the idea; e.g. G. Yang *et al.*, 2014; X. Yang *et al.*, 2021; but see Veresoglou *et al.*, 2020; Table 1).

Scenario C presents a special case of *Scenario B* and specifically describes fitness benefits in the form of a reduction in temporal variability exclusively under adverse conditions. A means by which plants could experience such a reduction in temporal variability under adverse conditions is if they can recover faster from environmental perturbations (i.e. have a higher resilience; Veresoglou *et al.*, 2020; Jia *et al.*, 2021b).

Remarkably, some of the expectations (those in *Scenario A* and *Scenario B*) of evolutionary bet-hedging are routinely captured in the existing literature because of the propensity of mycorrhizal
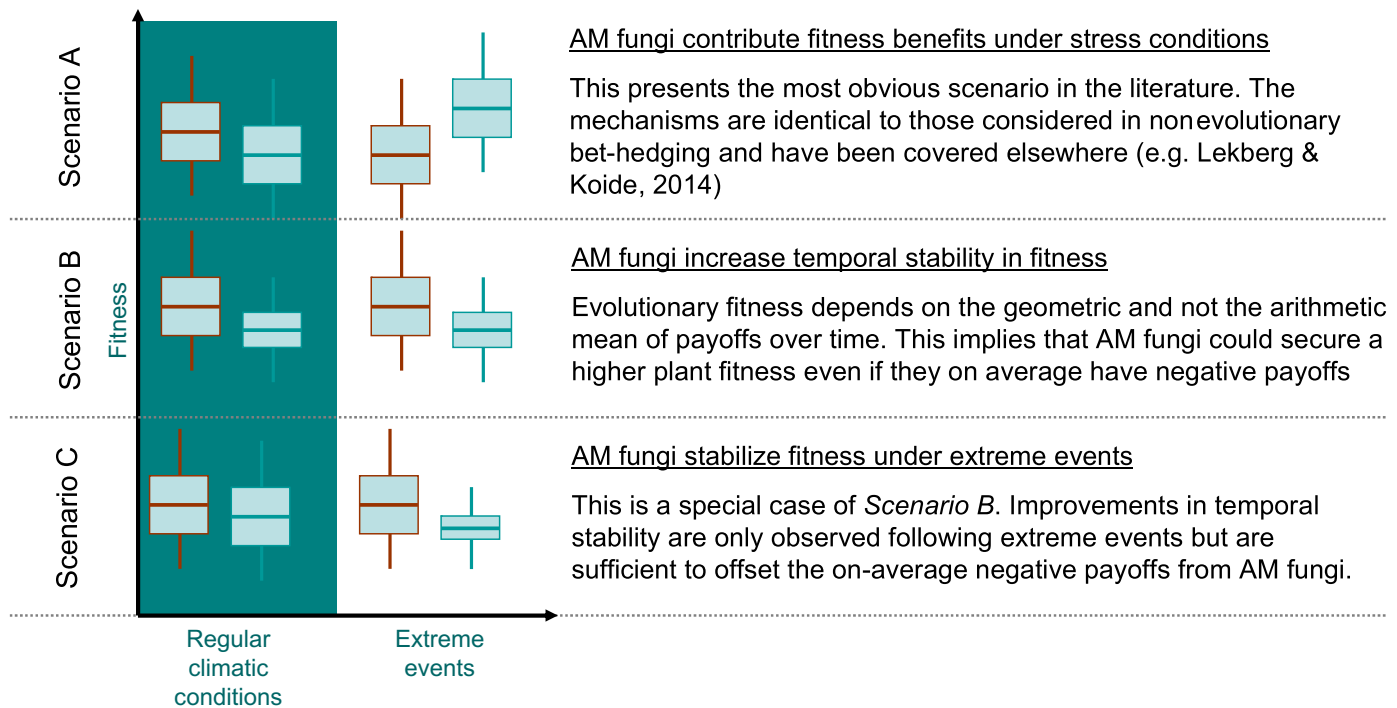
Fig. 1 Hypothetical fitness distributions (boxplots; the thick horizontal line represents the median fitness whereas the second and third quartiles are presented as the two edges of the overlying rectangle and the range is presented as a vertical line) of plants without a functional mycorrhiza (red boxplots) and plants with a functional mycorrhiza (green boxplots) under regular climatic conditions (shaded part of the figure) and following extreme events (nonshaded part of the figure) which could trigger an evolutionary bet-hedging strategy. We distinguish three scenarios under which arbuscular mycorrhizal (AM) fungi may induce an evolutionary bet-hedging. In *Scenario A*, plants offset negative payoffs from AM fungi under regular climatic conditions by better tolerating extreme climatic conditions. In *Scenario B*, plants associating with AM fungi may have a lower average fitness but experience an improved temporal stability in fitness. In *Scenario C*, the improvement in temporal stability only occurs under extreme environmental conditions (and could be expressed as improved resilience to extreme events) but is sufficiently strong to offset the lower average fitness gains.

Table 1 Comparative summary of existing studies, and our reanalysis of the Alternative Wheat and Fallow experiment, showing how arbuscular mycorrhizal (AM) fungi alter aspects of the temporal stability of plant host productivity and ecosystem functioning.

Study	Response variable	Effect of AM fungi on temporal variability (coefficient of variation of the response variable; a lower temporal variability would support *Scenario B*)	Effect of AM fungi on resistance	Effect of AM fungi on resilience (an improved resilience could support *Scenario C*)	Limitations
G. Yang *et al*. (2014)	Plant biomass	Increase			AM fungi were suppressed with benomyl, which can have nontarget effects and act in some cases as a fertilizer
Veresoglou *et al*. (2020)	Ecosystem respiration	Decline		Increase	Interpreting ecosystem respiration in short-term assays can be difficult
Jia *et al*. (2021a)	Plant biomass	No effects			AM fungi were suppressed with benomyl, which can have nontarget effects and act in some cases as a fertilizer
Jia *et al*. (2021b)	Multifunctionality following a drought manipulation		Increase	Increase	Unrealistically strong environmental perturbation (drought)
X. Yang *et al*. (2021)	Plant biomass	Increase			AM fungi were suppressed with benomyl, which can have nontarget effects and act in some cases as a fertilizer

ecologists to extensively invest in metaanalyses, using log-response ratios as effect sizes. Let us assume that w_i presents the relative fitness of a plant species that does not form mycorrhizas over generation i. Counterparts of the plant species that associate with mycorrhiza have a multiplicative relative fitness increment of λ_i (i.e. the inverse of an AM fungal response ratio – RR). The fitness of the plant species forming mycorrhiza, R_{AM}, compared to that of its nonmycorrhizal counterparts, R_{NM}, after n generations will be:

$$\overline{R}_{AM} = \left(\prod_1^n w_i \lambda_i\right)^{1/n} = R_{NM}\left(\prod_1^n \lambda_i\right)^{1/n} \qquad \text{Eqn 1}$$

which following a log transformation of both sides becomes:

$$\log \overline{R}_{AM} = \log \overline{R}_{NM} + \frac{1}{n}\sum_1^n (\log \lambda_i) = \log \overline{R}_{NM} - \overline{\log RR_{AM(i)}}$$

$$\text{Eqn 2}$$

It follows that a necessary and sufficient condition (in *Scenario A* and *Scenario B*) for AM fungi to benefit plants is that the average log response ratios of plant fitness in response to AM fungi be above zero. This is an expectation that has been routinely tested (even though mostly via procedures using weighting techniques) in numerous mycorrhizal metaanalyses (e.g. Treseder, 2004; Hoeksema *et al.*, 2010). Metaanalytical approaches also capture many of the abstractions (and thus biases) of the experimental procedures that are routinely used in mycorrhizal ecology, such as the unrealistic growth settings with nutrient-deficient sand, soil mixtures used for brief growth assays (Hoeksema *et al.*, 2010), and the use of plant biomass production as a good proxy of fitness (Younginger *et al.*, 2017). It would nevertheless be useful to further explore the degree to which we could take advantage of such metaanalyses to explore evolutionary bet-hedging as well as to develop approaches to discriminate between the two underlying mycorrhizal effects (i.e. growth stimulation and reduced temporal variability) on plant growth. Despite some preliminary studies providing evidence that points in this direction (e.g. Veresoglou *et al.*, 2020; Jia *et al.*, 2021b), it is not yet clear whether AM fungi additionally contribute to a higher resilience (*Scenario C*) in the systems where they occur, and this now represents a pressing topic in mycorrhizal ecology (e.g. Yang *et al.*, 2018).

Revisiting existing syntheses could probably quantify the variability of growth responses to mycorrhiza over iterative trials but, because it is difficult to reconstruct environmental conditions in the field, it probably cannot answer the question of whether eventually plants profit from an evolutionary bet-hedging. Finding appropriate settings to test the hypothesis of evolutionary bet-hedging is challenging. A promising avenue in palaeoecology is to reconstruct past distribution ranges of plants and assess how variable they have been over time (e.g. Gavin *et al.*, 2014): if biomass of AM-associating plants varies less with time than across non-AM-associating plants, this could be an indication of increased evolutionary fitness, which can then be compared with respective benefits from short-term experiments. Alternatively, it might be easy to use a space-for-time substitution approach (Johnson &

Miyanishi, 2008): for example, by monitoring the growth of plants over a range of settings, even outside their distribution range and assess whether the benefits (but also the respective temporal variability) gained from associating with AM fungi are systematically greater for any particular type of settings.

We developed the idea that associations with AM fungi could persist even if, for some hosts, such associations do not result in intermediate, short-term (i.e. in a single generation timespan) fitness gains. We can envisage two ways through which studying bet-hedging in AM systems has relevance to other disciplines. First, given that stability of food yield is an essential constituent of food security (Schmidhuber & Tubiello, 2007), it is worth exploring whether managing land to support arbuscular mycorrhiza promotes consistency in delivering ecosystem services. A key part of sustainable agricultural intensification is to improve management of soil biodiversity (e.g. Tilman *et al.*, 2011) and to this end it is important to explore any possible ways that arbuscular mycorrhiza could contribute (Rillig *et al.*, 2016). Second, arbuscular mycorrhiza could serve as a model system in exploring bet-hedging across other symbiotic systems. AM systems present some desirable features such as ubiquity in nature (Smith & Read, 2008) and a relative ease of assaying fitness benefits (at least in the form of pragmatic proxies) to plants through biomass production. Using AM associations as a model system could streamline the study of bet-hedging across mutualisms, reveal parallels to comparable systems that possibly experience bet-hedging, such as orchids (Shefferson *et al.*, 2003), and uncover the degree to which bet-hedging differs between symbiotic and nonsymbiotic systems because of coevolution (Hoeksema, 2010).

Author contributions

SDV conceived the idea and carried out the statistical analyses. SDV, DJ and MCR together wrote the manuscript. MM and GY reviewed and contributed valuable suggestions that improved the manuscript.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 An arithmetic simulation giving rise to *Scenario B* on evolutionary bet-hedging.

Methods S1 Annotated R code to reproduce Fig. S1.

Notes S1 Evolutionary fitness additionally depends on fitness variance.

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