

Letters

Fungal endophytes improve the performance of host plants but do not eliminate the growth/defence trade-off

A comment on Bastias *et al.* (2021) 'Fungal endophytes can eliminate the plant growth–defence trade-off'

In natural environments, organisms usually must cope with limiting resources and allocate these resources to priority biological processes, restricting others. This internal prioritisation of resources can give birth to trade-offs between different processes/responses due to resource constraints and/or to a physiological or biomechanical limitation (De Jong & Klinkhamer, 2005). In ecology, a trade-off corresponds to a reduction in one trait when another trait is increased and vice versa (Bazzaz & Grace, 1997; Grubb, 2016). Evidence for these trade-offs exists for different groups of organisms (Smith, 1976; Bazzaz & Grace, 1997; Kover & Clay, 1998; Molina-Montenegro *et al.*, 2012, 2020b). In plants, typical trade-offs include, but are not limited to, tolerance to shade vs drought (Sack, 2004; Niinemets & Valladares, 2006), growth rate vs mortality (Wright *et al.*, 2010; Bigler, 2016) and investment in vegetative growth vs defence (Herms & Mattson, 1992; Züst & Agrawal, 2017). In the latter case, a negative crosstalk in hormonal signalling may be the involved mechanism (Ballaré & Austin, 2019; Van Batselaar & Van den Ackerveken, 2020). However, when resource limitation is lifted and/or requirements for those resources are reduced, some trade-offs seem to vanish (Grubb, 2016).

It has been reported that plant–microorganisms mutualistic association can help host plants cope with biotic and abiotic stress (Acuña-Rodríguez *et al.*, 2020b; Liu *et al.*, 2020; Petipas *et al.*, 2021; Trivedi *et al.*, 2022). These studies have focused on how microorganisms, such as plant growth-promoting rhizobacteria (PGPR) and/or mycorrhizal and endophytic fungi, can improve environmental tolerance and resource acquisition in their host plants (Smith & Read, 2008; Molina-Montenegro *et al.*, 2020a). For example, evidence has shown that, in addition to their commonly known role enhancing the plant rates of resource acquisition (Newsham, 2011; Hill *et al.*, 2019), beneficial fungi can induce the expression of key functional genes in their host plants, affecting hormonal (e.g. jasmonic acid) and biochemical pathways (i.e. related to defence alkaloids such as loline and peramine)

resulting in higher growth and/or higher tolerance to biotic stress (Rodríguez & Redman, 2008; Roberts & Lindow, 2014; Bastías *et al.*, 2017). Therefore, when plants associated with beneficial microorganisms are stressed, they may not experience such pressure in the same fashion as nonsymbiotic plants (Acuña-Rodríguez *et al.*, 2020a). Considering this, commonly observed trade-offs might disappear in symbiotic plants. Interestingly, some mutualistic fungi seem to induce increases in both growth and resistance/tolerance to herbivores and pathogens (Bennett *et al.*, 2006; Pieterse *et al.*, 2014); therefore, a plant associated with them may then have simultaneously high performances on survival, growth and defence. This is the case of some grass species that associate with *Epichloë* spp., a group of fungal endophytes that can produce defence alkaloids and stimulate hormone pathways related to growth (Dupont *et al.*, 2015; Schmid *et al.*, 2017). Nevertheless, empirical evidence is still needed to correctly address if the growth/defence trade-off actually disappears (please refer to Grubb, 2016).

In a recent paper in *New Phytologist*, Bastias *et al.* (2021), by studying the grass–*Epichloë* model, proposed that fungal endophytes can eliminate the growth/defence trade-off. In particular, they suggested that an increased resistance to herbivores would not result in a reduced growth in grasses associated with *Epichloë*. This was approached by reviewing the role of these endophytes in increased defence and growth (mechanisms), a meta-analysis on reported effects of *Epichloë* on plant defences and growth (bibliographical data), and a generalized linear model (GLM) analysing the relation between the changes in growth and resistance to herbivores as a consequence of the presence of *Epichloë* in various host plants, which also included previously published data. The GLM showed a lack of a significant negative slope when plotting plant growth gain and resistance gain differentials between symbiotic and asymbiotic plants, which they interpreted as a lack of trade-off (but please refer to Grubb, 2016). Although, the study of Bastias *et al.* (2021) is a significant contribution to the understanding of the overall effect of endophytes on the growth/defence trade-off, we think their conclusions regarding the elimination of the trade-off should be taken with caution and need a deeper examination. In their meta-analysis, Bastias *et al.* (2021) showed that *Epichloë* is capable of triggering positive responses on the growth and defence traits of various host plants. Moreover, in 31 study cases that simultaneously measured growth and resistance to herbivory in E– (asymbiotic) and E+ (symbiotic) plants (Ueno *et al.*, 2016), they showed no relation between both responses. Nevertheless, most of their conclusions regarding the elimination of the growth/defence trade-off rely, as stated above, on their nonsignificant GLM analysis, which from our point of view is not enough to make that claim. The main reason behind our rationale is the lack of an unequivocal demonstration of a growth/defence trade-off among nonsymbiotic (E–) plants in the studied species (which is supposed to be eliminated). This is mainly because their study lacked a true control (E– plants) for comparison and only showed experimental data for E+ plants.

Therefore, we consider that it is crucial to establish the relationship between growth and defence (i.e. the existence of the trade-off) in nonsymbiotic individuals from each studied plant species before addressing how endophytes could disrupt any trade-off. This is particularly relevant when, as for Bastias *et al.* (2021), instead of individual trait values, the effect size of the endophyte presence per plant species is used as the modelled variable. In this sense, each point represents an averaged response of one plant species–*Epichloë* study case pair with unknown variability. Therefore, although this approach might provide an idea about changes in resource allocation in the presence of endophytes, it does not necessarily involve the existence of a subadjacent compromise between growth and defence among E– individuals. This might be particularly true for those plant–host cases with effect size values close to zero in both x and y axes. For these points we might only suppose that they maintain their allocation priorities despite their symbiotic state (E+ or E–). However, as there is no biological effect of the fungal endophyte in the referred priorities, the existence of the trade-off is indeed, unclear. In this sense, as suggested by Grubb (2016), to properly test a trade-off it is required to evaluate if the

endophyte presence in plants would not result in a reduction in one response when the other is increased. This should be done by plotting growth and defence responses (not the differential effects) and testing if there is a significant negative relationship (negative slope) between those responses in both E– and E+ plants, separately.

To test the hypothesis proposed by Bastias *et al.* (2021), which is a suppression of the growth/defence trade-off due to the positive effects of endophytes on plant resource status, we gathered data that evaluated this compromise between growth and defence in nonsymbiotic (E–) and endophyte symbiotic plants (E+) of *Hordeum murinum* ('wall barley'), a common grass from South Patagonia that is commonly infested by the aphid *Rhopalosiphum padi* (Nieto-Nafría *et al.*, 2016) (for details please refer to Supporting Information Methods S1). The beneficial effect of endophyte symbionts has been previously demonstrated on some plants under abiotic stress (Molina-Montenegro *et al.*, 2015, 2016, 2020a; Hereme *et al.*, 2020; Torres-Díaz *et al.*, 2021). From these studies, we assume that the experimental design (E+ and E– plants) effectively disrupts any potential positive effect of the plant–

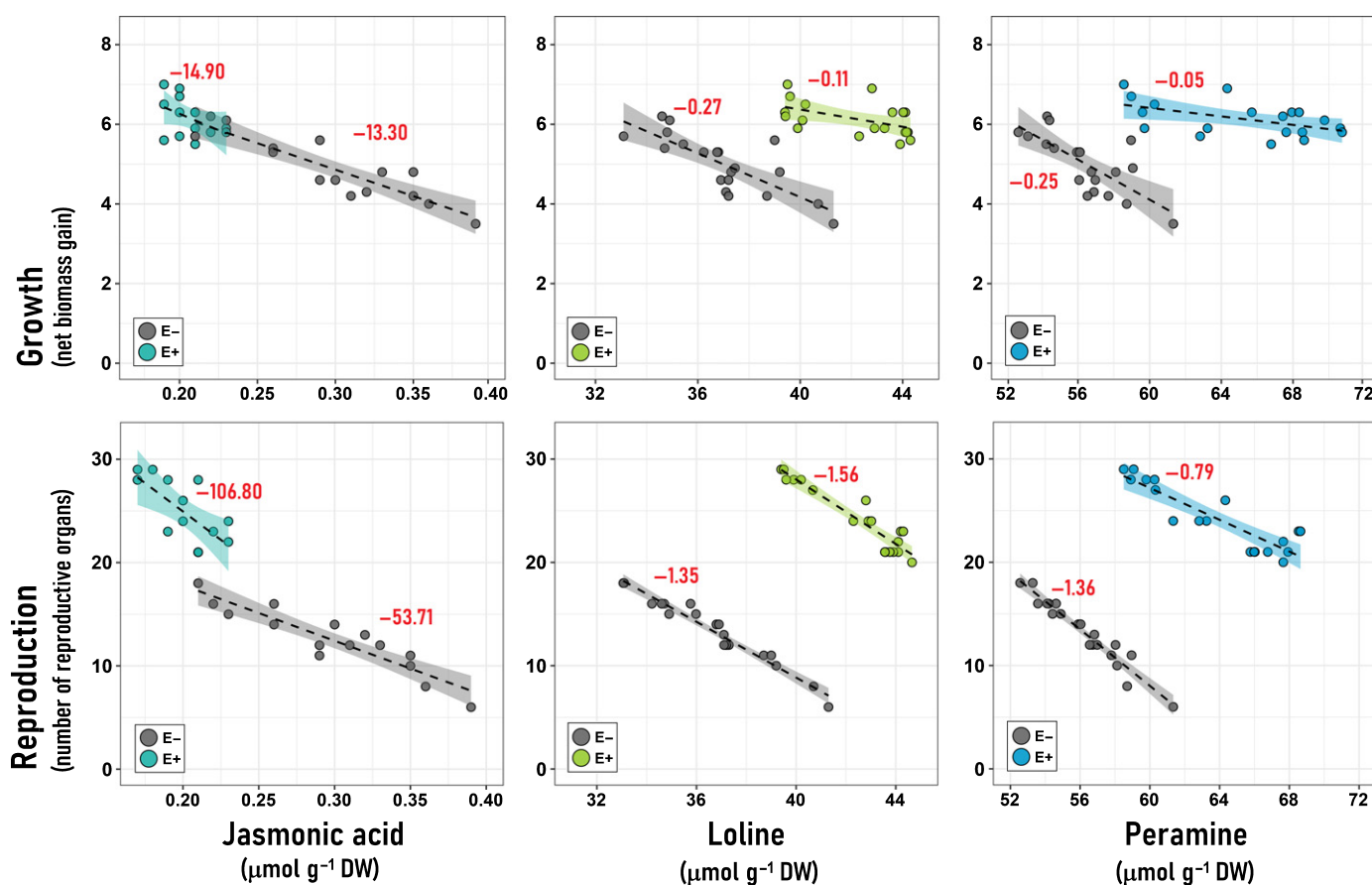


Fig. 1 Relationship between two fitness-related traits (net biomass gain and number of reproductive organs) and three plant biomarkers related with herbivory stress (jasmonic acid, loline and peramine) in *Hordeum murinum* (Poaceae) from Southern Patagonia. The fitted model trends are shown for both nonsymbiotic plants (E–, in grey) and endophyte inoculated plants (E+, in colour) together with their 95% confidence intervals (shaded area along the respective fitted line). For the jasmonic acid data $n = 14$ and 15 plants (E+ and E–, respectively) while for the loline and peramine estimation $n = 20$ for both E– and E+ groups. The slope value for each linear model is shown in red next to the respective regression line if significant (i.e. different from zero). As in all cases, both regression lines were significantly distinct from zero, a comparison of the linear trend shown by E+ and E– groups was performed in each case (please refer to Supporting Information Tables S1, S2).

microbe symbiosis in E− plants. We performed independent linear models using data from plants with and without fungal endophytes (E+ and E− plants) in which two fitness proxies (growth and reproductive investment) and three well known biochemical responses to herbivory were assessed. Specifically, we measured jasmonic acid as a hormonal response to herbivory, and loline and peramine as defence-related alkaloids (please refer to Rodríguez & Redman, 2008; Ren & Dai, 2012; Roberts & Lindow, 2014; Bastías *et al.*, 2017). In this way, we assessed the relationship between the capacity of plants to tolerate herbivory in both symbiotic (E+) and nonsymbiotic (E−) plants, and their fitness investment (Table S1). In addition, we tested separately for each group the significance of their slopes (the existence of a trade-off) and, in cases where both E+ and E− regressions resulted either significant or not-significant, a further test of covariance between the linear trends from E+ and E− plants was performed.

We found a significantly negative relationship between both fitness proxies and all the assessed defence traits in E− and E+ plants (Fig. 1). This supports not only the existence of a trade-off between growth/reproduction and defence to herbivory without the participation of the plant endophytes, but also that the trade-off persists, at least in this species, in symbiotic (E+) plants (Fig. 1; Table S1), contrary to the observation of Bastías *et al.* (2021). Certainly, a greater average production of stress-related chemical compounds in E+ compared with E− plants confirmed the known positive role of the fungal endophytes (Fig. 1). However, despite their positive role, the within-biomarker slope comparisons (E− vs E+) was only significantly reduced in half of the analysed cases (growth vs loline, growth vs peramine and reproduction vs peramine, Table S2), however without becoming nonsignificant, therefore maintaining the trade-off. Therefore, no evidence of the expected trade-off elimination predicted by Bastías *et al.* (2021) was found in our study system. Although, we did not test the effect of the symbiosis using *Epichloë* endophytes, and comparisons should be taken with some caution, our dataset indicated that the beneficial effects of endophytes are ecologically relevant, but are not sufficient to break down the compromise between fitness (growth or reproduction) and defence responses in a grass such as *H. murinum*.

We are still far from understanding all possible outcomes in the plant–endophyte interactions, especially if they are independent of ecological context, or instead particularly related to a given environmental stress/interaction. In addition, further surveys are needed to re-evaluate the cases in which trade-offs seem to be broken by plant–endophytes symbiosis and unravel the underlying mechanisms. Our Letter identifies promising avenues to promote a scientific debate about the extent of the positive effects exerted by endophytes in their host plants.


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Author contributions

CA, ISA-R and MAM-M planned and designed the research. ISA-R, CT-D and MAM-M performed experiments and analysed data. CA, ISA-R, CT-D and MAM-M wrote the manuscript.


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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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References

- Acuña-Rodríguez IS, Galán A, Torres-Díaz C, Atala C, Molina-Montenegro MA. 2020a. Fungal symbionts enhance N-uptake for Antarctic plants even in non-N limited soils. *Frontiers in Microbiology* 11: 2660.
- Acuña-Rodríguez IS, Newsham KK, Gundel PE, Torres-Díaz C, Molina-Montenegro MA. 2020b. Functional roles of microbial symbionts in plant cold tolerance. *Ecology Letters* 23: 1034–1048.
- Ballaré CL, Austin AT. 2019. Recalculating growth and defense strategies under competition: key roles of photoreceptors and jasmonate. *Journal of Experimental Botany* 70: 3425–3434.
- Bastías DA, Gianoli E, Gundel PE. 2021. Fungal endophytes can eliminate the plant growth–defence trade-off. *New Phytologist* 230: 2105–2113.
- Bastías DA, Martínez-Ghersa MA, Ballaré CL, Gundel PE. 2017. *Epichloë* fungal endophytes and plant defenses: not just alkaloids. *Trends in Plant Science* 22: 939–948.
- Bazzaz FA, Grace J. 1997. *Plant resource allocation*. San Diego, CA, USA: Academic Press, 303.
- Bennett AE, Alers-García J, Bever JD. 2006. Three-way interactions among mutualistic mycorrhizal fungi, plants, and plant enemies: hypotheses and synthesis. *American Naturalist* 167: 141–152.
- Bigger C. 2016. Trade-offs between growth rate, tree size and lifespan of mountain pine (*Pinus montana*) in the Swiss national park. *PLoS ONE* 11: e015402.

- De Jong T, Klinkhamer P. 2005. *Evolutionary ecology of plant reproductive strategies*. Cambridge, UK: Cambridge University Press.
- Dupont PY, Eaton CJ, Wargent JJ, Fechtner S, Solomon P, Schmid J, Day RC, Scott B, Cox MP. 2015. Fungal endophyte infection of ryegrass reprograms host metabolism and alters development. *New Phytologist* 208: 1227–1240.
- Grubb PJ. 2016. Trade-offs in interspecific comparisons in plant ecology and how plants overcome proposed constraints. *Plant Ecology & Diversity* 9: 3–33.
- Herme R, Morales-Navarro S, Ballesteros G, Barrera A, Ramos P, Gundel PE, Molina-Montenegro MA. 2020. Fungal endophytes exert positive effects on *Colobanthus quitensis* under water stress but neutral under a projected climate change scenario in Antarctica. *Frontiers in Microbiology* 11: 264.
- Hermis DA, Mattson WJ. 1992. The dilemma of plants: to grow or defend. *Quarterly Review of Biology* 67: 283–335.
- Hill PW, Broughton R, Bougoure J, Havelange W, Newsham KK, Grant H, Murphy DV, Clode P, Ramayah S, Marsden KA *et al.* 2019. Angiosperm symbioses with non-mycorrhizal fungal partners enhance N acquisition from ancient organic matter in a warming maritime Antarctic. *Ecology Letters* 22: 2111–2119.
- Kover PX, Clay K. 1998. Trade-off between virulence and vertical transmission and the maintenance of a virulent plant pathogen. *American Naturalist* 152: 165–175.
- Liu H, Brettell LE, Qiu Z, Singh BK. 2020. Microbiome-mediated stress resistance in plants. *Trend in Plant Sciences* 25: 733–743.
- Molina-Montenegro MA, Acuña-Rodríguez IS, Torres-Díaz C, Gundel PE, Dreyer I. 2020a. Antarctic root endophytes improve physiological performance and yield in crops under salt stress by enhanced energy production and Na⁺ sequestration. *Scientific Reports* 10: 5819.
- Molina-Montenegro MA, Baldelomar M, Atala C, Torres-Díaz C. 2020b. A tradeoff between fitness-related traits mask facilitation in a semiarid ecosystem. *Oikos* 129: 1196–1203.
- Molina-Montenegro MA, Cleland EE, Watts SM, Broitman BR. 2012. Can a breakdown in competition-colonization tradeoffs help explain the success of exotic species in the California flora? *Oikos* 121: 389–395.
- Molina-Montenegro MA, Osés R, Torres-Díaz C, Atala C, Núñez MA, Armas C. 2015. Fungal endophytes associated with roots of nurse cushion species have positive effects on native and invasive beneficiary plants in an alpine ecosystem. *Perspectives in Plant Ecology, Evolution and Systematics* 17: 218–226.
- Molina-Montenegro MA, Osés R, Torres-Díaz C, Atala C, Zurita-Silva A, Ruiz-Lara S. 2016. Root-endophytes improve the ecophysiological performance and production of an agricultural species under drought condition. *AoB Plants* 8: lw062.
- Newsham KK. 2011. A meta-analysis of plant responses to dark septate root endophytes. *New Phytologist* 190: 783–793.
- Nieto-Nafria JM, Fuentes-Contreras E, Castro-Colmenero M, Aldea-Piera M, Ortego J, Mier-Durante MP. 2016. Catálogo de los áfidos (Hemiptera, Aphididae) de Chile, con plantas hospedadoras y distribuciones regional y provincial. *Graellsia* 72: e050.
- Niinemets Ü, Valladares F. 2006. Tolerance to shade, drought, and waterlogging of temperate Northern Hemisphere trees and shrubs. *Ecological Monographs* 76: 521–547.
- Petipas RH, Geber MA, Lau JA. 2021. Microbe-mediated adaptation in plants. *Ecology Letters* 24: 1302–1317.
- Pieterse CMJ, Zamioudis C, Berendsen RL, Weller DM, Van Wees SCM, Bakker PAHM. 2014. Induced systemic resistance by beneficial microbes. *Annual Review of Phytopathology* 52: 347–375.
- Ren CG, Dai CC. 2012. Jasmonic acid is involved in the signaling pathway for fungal endophyte-induced volatile oil accumulation of *Atractylodes lancea* plantlets. *BMC Plant Biology* 12: 128.
- Roberts E, Lindow S. 2014. Loline alkaloid production by fungal endophytes of Fescue species select for particular epiphytic bacterial microflora. *ISME Journal* 8: 359–368.
- Rodríguez R, Redman R. 2008. More than 400 million years of evolution and some plants still can't make it on their own: plant stress tolerance via fungal symbiosis. *Journal of Experimental Botany* 59: 1109–1114.
- Sack L. 2004. Responses of temperate woody seedlings to shade and drought: do trade-offs limit potential niche differentiation? *Oikos* 107: 110–127.
- Schmid J, Day R, Zhang N, Dupont PY, Cox MP, Schardl CL, Minards N, Truglio M, Moore N, Harris DR *et al.* 2017. Host tissue environment directs activities of an *Epichloe* endophyte, while it induces systemic hormone and defense responses in its native perennial ryegrass host. *Molecular Plant-Microbe Interactions* 30: 138–149.
- Smith CC. 1976. When and how much to reproduce - trade-off between power and efficiency. *American Zoologist* 16: 763–774.
- Smith S, Read D. 2008. *Mycorrhizal symbiosis*. London, UK: Academic Press, 800.
- Torres-Díaz C, Valladares MA, Acuña-Rodríguez IS, Ballesteros GI, Barrera A, Atala C, Molina-Montenegro MA. 2021. Symbiotic interaction enhances the recovery of endangered tree species in the fragmented Maulino forest. *Frontiers in Plant Science* 12: 663017.
- Trivedi P, Batista BD, Bazany KE, Singh BK. 2022. Plant microbiome interactions under a changing world: responses, consequences, and perspective. *New Phytologist* 234: 1951–1959.
- Ueno AC, Gundel PE, Omacini M, Ghersa CM, Bush LP, Martínez-Ghersa MA. 2016. Mutualism effectiveness of a fungal endophyte in an annual grass is impaired by ozone. *Functional Ecology* 30: 226–232.
- Van Butselaar T, Van den Ackerveken G. 2020. Salicylic acid steers the growth-immunity tradeoff. *Trends in Plant Science* 25: 566–576.
- Wright SJ, Kitajima K, Kraft NJB, Reich PB, Wright IJ, Bunker DE, Condit R, Dalling JW, Davies SJ, Díaz S *et al.* 2010. Functional traits and the growth-mortality trade-off in tropical trees. *Ecology* 91: 3664–3674.
- Züst T, Agrawal AA. 2017. Trade-offs between plant growth and defense against insect herbivory: an emerging mechanistic synthesis. *Annual Review of Plant Biology* 68: 513–534.

Supporting Information

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

Methods S1 Statistical analysis, biological material and experimental design performed.

Table S1 Linear regression analyses performed to evaluate the existence of trade-offs between growth or reproduction and herbivory resistance proxies.

Table S2 Analysis of variance for fitness proxies and biomarkers of herbivory, relative to the presence of a fungal endophyte.

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