

# Symbiotically modified organisms: nontoxic fungal endophytes in grasses

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**We propose that symbiotically modified organisms (SMOs) should be taken into account in sustainable agriculture. In this opinion article, we present the results of a meta-analysis of the literature, with a particular focus on the potential of SMOs in forage and turf grass production, to determine the impact of endophytes in grasses on livestock, the grassland ecosystems, and associated environments. SMOs can be incorporated into breeding programs to improve grass yield, resistance to pests and weeds, and forage quality for livestock by decreasing the level of toxic alkaloids. However, the benefits of these selected grass–endophyte symbiota appear to be highly dependent on grass cultivar, fungal strain, and environmental conditions, requiring a comprehensive understanding of the genetic bases and phenotypic plasticity of the traits of the plant–microbe unit in different environments.**

## Heritable SMOs in plant production

Meeting the increasing demand for food is of prime importance as the human population continues to grow globally [1–3]. Consequently, agriculture has become industrialized, and more land has been assigned to crop and forage production, while pasture-based livestock production has been relocated to marginal areas [2,3]. This development has involved innovations in breeding technologies, machinery, and farming methods, including increased use of synthetic agrochemicals, such as fertilizers, pesticides, and herbicides [3,4]. However, concerns over the associated environmental risks and sustainability of industrialized agriculture have provoked the need for more environmentally friendly agricultural practices [3,5]. Plant breeding programs to improve plant productivity, nutrient- and water-use efficiency, tolerance to pests, and forage quality for livestock are key to meeting the increasing demand for food using sustainable agriculture [3,5,6]. The selection of desirable plant traits for agricultural purposes has been practiced for thousands of years since the domestication of the first agricultural plants [7]. Today, breeding processes

range from traditional selective breeding to the use of new molecular tools that enable genetic selection and/or manipulation, which involve genetic engineering techniques, such as knockout and deletion of genes, and horizontal gene transfer between species in genetically modified organisms (GMOs) (see [Glossary](#)) [5,8–10].

Here, we explore the feasibility of harnessing the positive effects of symbiotic microorganisms in plant breeding and production [11,12]. The potential of symbioses depends on the life-history traits of the host and the symbiont, which largely determine the manageability and the predictability of the symbiotum. For example, although growth-promoting bacteria, nitrogen-fixing bacteria, and mycorrhizal fungi are used in agriculture, crops need to be repeatedly re-inoculated with the symbiont, because these free-living microorganisms do not form persistent symbioses [11,13]. By contrast, a heritable symbiosis with higher persistence potentially provides a more reliable and more labor- and cost-effective means for plant breeding and production. One of the best-studied examples of a heritable symbiosis is between grasses and systemic fungal endophytes. In this symbiosis, the systemic fungus subsists entirely on the host and is vertically transmitted from the mother plant to the offspring via the host seed. Thus, the fitness of the fungus largely depends on the fitness of the host plant; the production of different mycotoxins that endow plants with protection against herbivores is considered the currency of the symbiosis. In addition to increasing the resistance of the plant to herbivores and pathogens, systemic endophytes often increase plant vigor and tolerance to various environmental conditions; thus, grass endophytes are generally labeled as strong plant mutualists [14–21].

Although grass–endophyte symbioses are ideal research systems for testing theoretical ecological and coevolutionary questions, they have been, and still are, an economically important agronomic issue. During the 1970s, livestock disorders caused by fungal alkaloids in the agronomic grasses tall fescue (*Schedonorus phoenix*; synonym *Festuca arundinacea*) and perennial ryegrass (*Lolium perenne* L.) led to the discovery that animal toxicosis is related to systemic grass–endophyte symbioses [22–24]. Alkaloids of the ergot family and lolitrem B synthesized by wild fungal strains of *Neotyphodium coenophialum* and *Neotyphodium lolii* in tall fescue and perennial ryegrass, respectively, were found to be responsible for fescue toxicosis and

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## Glossary

**Alkaloid:** generically, a secondary nitrogen-rich compound produced by any plant, animal, fungus, or bacteria that may have toxic or pharmacological effects on animals. In the context of this article, alkaloids are fungal compounds that affect the interaction of host plants with their enemies, namely herbivores. They are considered defenses because they usually improve plant fitness and affect negatively herbivore performance. Four well-known groups of alkaloids in grass–endophyte symbioses are lolines, peramine, ergot alkaloids, and lolitrem.

**Animal toxicosis:** disease caused by the poisoning effect of consuming a toxic compound in food. In this article, the main animal toxicoses referred to in domestic animals are ergotism and ryegrass stagger, caused by consuming *Schedonorus phoenix* and *Lolium perenne* infected by *Neotyphodium coenophialum* and *Neotyphodium lolii*, respectively. The poisoning compounds behind such effects are ergovaline and lolitrem b for *S. phoenix*–*N. coenophialum* and *L. perenne*–*N. lolii*, respectively.

**Endophyte infected plant (E+):** a plant that is naturally infected by a systemic fungal endophyte. These fungi are also referred to as ‘wild type’ or ‘standard’ endophytes.

**Ergots:** refer generically to a complex group of fungal alkaloids produced by the fungus *Claviceps purpurea* and related fungi, known for causing intoxications in vertebrates, including humans (ergotism). Within the three major groups (clavines, amides of lysergic acid, and ergopeptines), ergovaline and lysergic acid amide are among the most abundant and active found in the grass–endophyte symbiosis.

**Ergovaline:** the main ergot alkaloid associated with disorders in livestock grazing on pastures of *S. phoenix* infected by the fungal endophyte *N. coenophialum*.

**Genetically modified organism (GMO):** an organism whose genetic material has been modified by transgenesis.

**Lolines:** a group of fungal alkaloids (saturated pyrrolizidines) that may be toxic and effective feeding deterrents for insects. The most common forms are: *N*-formyllooline, *N*-acetyllooline, *N*-acetyl norlooline, and *N*-norlooline.

**Lolitrem:** indole diterpene alkaloids that are tremorgenic neurotoxins (i.e., produce muscle tremor) in animals. Lolitrem b is the fungal alkaloid responsible for ryegrass stagger disease in animals grazing on *L. perenne* colonized by the fungal endophyte *N. lolii*.

**Manipulatively endophyte free plant (ME–):** a plant that has been manipulated to remove its natural fungal endophyte by fungicide (from seedlings) or heat (from seed) treatment. Although there are also naturally endophyte free (E–) plants in grass populations, endophyte removal is a common experimental treatment to study the effects of symbiosis on host plants (by comparing, for example, E+ with ME– plants).

**Mycotoxin:** any fungal compound (in this article, alkaloid) with negative (poisoning) effects on any aspect of the performance of the invertebrate or vertebrate animals. Mycotoxins may act as metabolic toxins or feeding deterrents.

**Paratransgenesis:** modification of the phenotype of an organism by genetic transformation of its symbiotic organisms; for example, when knockout gene endophytes are inoculated into a grass cultivar [SMO (GMO)].

**Peramine:** isolated pyrrolopyrazine alkaloid of fungal origin that is known to deter some invertebrates from consuming the E+ plants.

**Plant infected with selected endophyte (SE+):** a plant that has been inoculated with an endophyte selected from the naturally occurring endophytes within the species-specific host, but that does not produce ergot in the case of *S. phoenix*, or lolitrem b in the case of *L. perenne*. Therefore, such endophytes should be nontoxic to livestock. They can be inoculated into E– cultivars or into E+ cultivars by replacing a toxic fungal strain within the same host species. These selected fungal endophytes have been termed as ‘novel’, ‘safe’, or ‘non-toxic’ endophytes.

**Symbiotically modified organism (SMO):** an organism whose phenotype has been modified through the manipulation (introduction or change) of a symbiotic microorganism. If it is vertically transmitted from host to offspring, these symbionts can be considered as heritable host characters in breeding.

**Symbiotum (plural symbiota):** an established plant–microbe interaction.

**Transgenesis:** introduction of a novel gene into the genome of an organism.

ryegrass staggers syndrome. The importance of systemic endophytes was readily accepted as probably being ubiquitous. They are found in at least 80 genera and 300 grass species [25], many of which represent a significant part of the natural food for wild mammalian grazers and forage for livestock and dairy cows in cultivated pastures and grasslands globally [26]. In addition to the agricultural arena, these grasses are widely used as turf in recreational areas and landscaping. Given that economic losses due to tall

fescue infected with endophytic fungi that produce ergot alkaloids have been estimated at US\$609 million annually in the USA alone [27,28], cultural practices to reduce toxicosis have been developed and applied (e.g., [27,29]). In addition, for more than two decades, particularly in the USA and New Zealand, grass breeders have been intensively researching methods of incorporating into forage breeding programs systemic endophytes that do not produce the toxic alkaloids [30,31].

Different methodologies, from conventional selective breeding to modern biotechnological engineering, have been applied to control disadvantages and harness the benefits of endophytes in grass-breeding programs [31,32]. For example, breeders may select (i) either naturally endophyte-infected (hereafter E+) or naturally endophyte-free (hereafter E–) high-performing plants to produce new varieties; (ii) manipulate the endophyte infection status of the germplasm by either re-inoculating elite cultivars with selected endophytes (hereafter SE+) or by removing the natural wild endophyte from cultivars (hereafter ME–); or (iii) use genetic engineering techniques to manipulate the endophyte [31–33].

In this opinion article, we test by meta-analysis (see the [supplementary material online](#)) whether the available literature supports the presumed feasibility of a human-made host–endophyte symbiosis to increase forage yield and quality for livestock without compromising ecological and environmental factors. Specifically, we focused on those fungal endophytes that have been selected and inoculated into forage cultivars producing no toxic alkaloids to livestock (SE+) but that do produce alkaloids related to pest resistance (lolines and peramine). Because manipulating the symbiosis for breeding purposes is analogous to using genetic engineering to add new traits to create GM plants, we propose that novel associations between a grass and an endophyte and other plant–microbe symbiota that have been established by humans be referred to as SMOs. However, compared with GMOs, SMOs will probably be more readily accepted by the public because they operate on natural species-specific symbiosis without any intervention at the genetic or molecular level. Nevertheless, similar to the situation with GMOs, uncertainties with regard to economically measurable deliverables for end-users in the agribusiness and possible ecological risks associated with SMOs should not be ruled out, and the successful use of SMOs will require a more comprehensive understanding of the genetic bases and the phenotypic plasticity of traits of the plant–microbe unit in different environments.

## Improving forage production by fungal endophytes

To avoid animal toxicosis, the viability of the endophyte is generally reduced by storing the cultivar for a long period of time, or by subjecting the seeds to fungicide or heat treatments [34,35]. However, using these seeds is not necessarily trouble free. On a large scale, these methods are often found to be less effective because a proportion of seeds may retain viable endophytes [35,36], which enable the competitively superior endophyte-infected grasses to overtake the pastures within a few years [37–40]. This process can be exacerbated by any viable endophyte-infected seeds remaining in the soil bank [41,42]. Furthermore, lower performance is

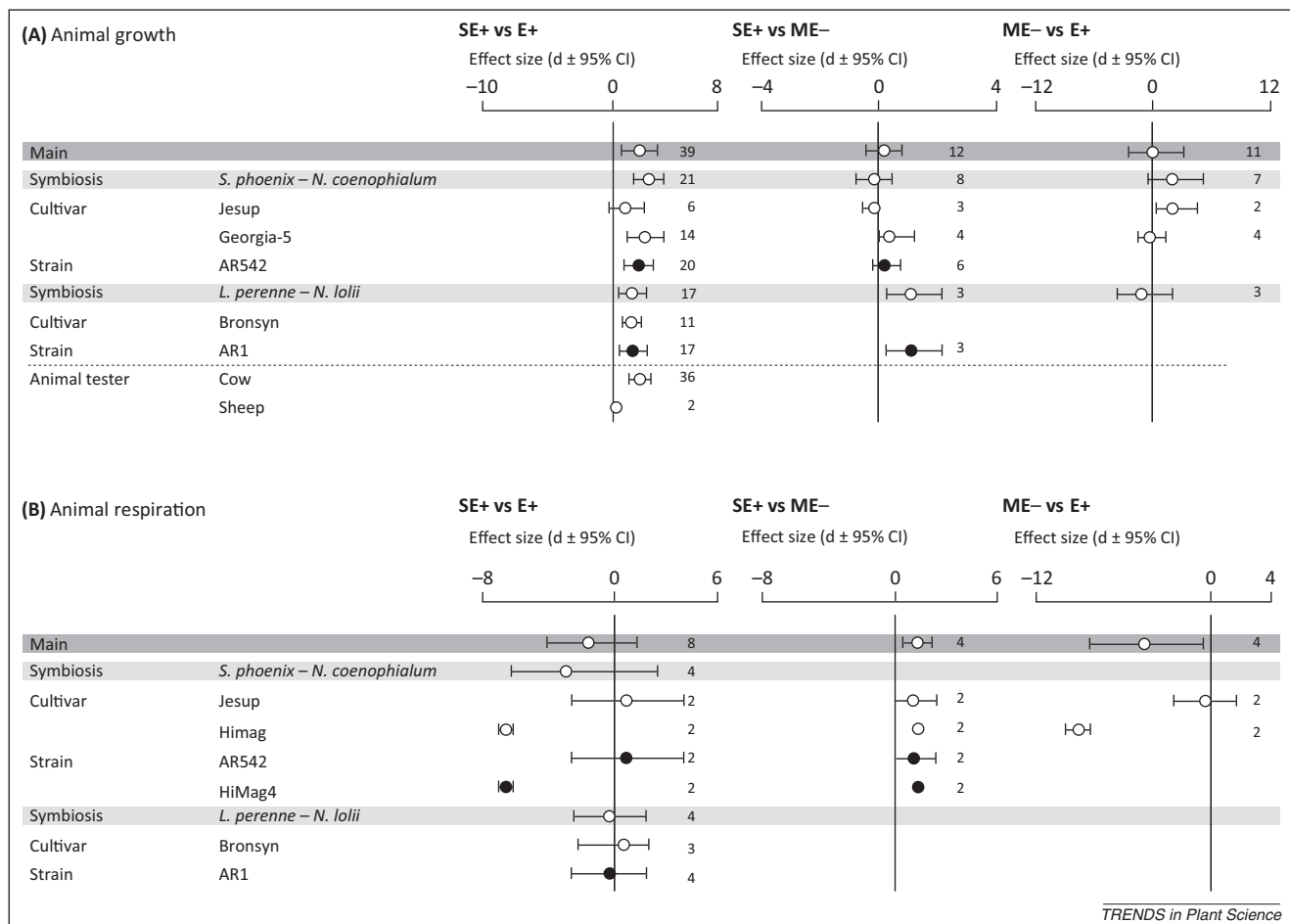
commonly detected in plants that have been manipulated to be endophyte-free plants, because endophytes can improve host plant fitness directly by increasing plant growth and reproduction, or indirectly by conferring resistance to insects and pathogens as well as to various abiotic stress factors, such as drought and heavy metals [17,18,43,44].

To boost forage grass-breeding programs, 'endophyte technologies' that involve manipulating the symbiosis by introducing selected endophyte strains into E- grass cultivars, or by replacing toxic fungal strains with reputed 'nontoxic' ones, have been deployed with high expectations that cultivars will combine the advantages of the high persistence of endophyte-infected plants with the beneficial attributes on animal health of E- plants [31–33]. Several grass varieties infected by selected endophytes have already been commercialized in the USA, New Zealand, Australia, and Uruguay, but the species are limited to tall fescue and perennial ryegrass and the performance and stability of the varieties have yet to be evaluated in real forage production [31,33].

#### Minimizing the risks of livestock disorders by selected endophytes

Our meta-analysis of the literature suggests that SE+ can improve forage quality (i.e., reduce the risk of animal

toxicosis) compared with E+ forage. Livestock consume more on SE+ plant cultivars than on E+ plants [overall effects on consumption:  $n = 10$ , effect size = 2.240, 95% confidence interval (CI) = 1.313, 3.606], but consumption was not different compared with ME- plants (overall effects on consumption:  $n = 9$ , effect size = -0.729, 95% CI = -1.469, 0.087). Similarly, growth was higher in animals that grazed over SE+ than over E+, whereas it was not different between animals grazing over SE+ or over ME- (Figure 1A). This pattern was observed not only for the *S. phoenix*–*N. coenophialum* symbiosis, but also for the *L. perenne*–*N. lolii* symbiosis. Animal growth was not significantly higher in tall fescue cultivar 'Jesup' and in the case of sheep. However, the magnitude of effect size did not vary among cultivars ( $Q_b = 2.464$ ,  $P = 0.482$ ,  $df = 3$ ) or between cow and sheep (animal tester;  $Q_b = 1.023$ ,  $P = 0.312$ ,  $df = 1$ ) (Figure 1A). The Rosenthal fail-safe numbers for the comparison between SE+ and E+ on consumption [Symbiosis:  $75.6 > 5 \cdot (10) + 10$ ] and growth [Symbiosis:  $300.5 > 5 \cdot (38) + 10$ ] resulted in higher numbers relative to that of sample size, indicating unbiased and reliable estimation of the effects. These results were also unbiased at the level of cultivar, strain, and animal tester, indicating that the analyses were sufficiently robust (Table S2A in the supplementary material online).



**Figure 1.** The effects of novel associations between host grasses and fungal endophytes on livestock. Mean effect sizes (d) and confidence intervals (CI) of the comparisons between SE+ and E+ plants, SE+ and ME- plants, and ME- and E+ plants to show the effects of selected endophytes on (A) animal growth and (B) animal respiration. Within each species-specific symbiotic association (symbiosis), white symbols represent cultivars and black symbols represent fungal strains. The number of studies is indicated to the right of each value.

The animal consumption of SE+ or ME– forage was not different at the level of symbiosis, cultivar, strain, and animal tester (data not shown). Although animal growth seemed to be positively affected by SE+ in perennial ryegrass but not in tall fescue (Figure 1A), this effect was not statistically significant for symbiosis ( $Q_b = 2.727$ ,  $P = 0.098$ ,  $df = 1$ ), cultivar ( $Q_b = 4.148$ ,  $P = 0.126$ ,  $df = 2$ ), and strain ( $Q_b = 2.677$ ,  $P = 0.102$ ,  $df = 1$ ). Furthermore, the consumption of ME– forage was higher compared with E+ forage ( $n = 9$ , effect size = 2.899, 95% CI = 1.659, 4.866), an effect that was significantly robust as indicated by the Rosenthal fail-safe number (Symbiosis:  $68.1 > 5 \cdot (9) + 10$ ) (Table S2A in the supplementary material online). However, these differences in consumption were not translated into animal growth (Figure 1A) (Table S1C in the supplementary material online).

Given that the SE+ cultivars examined were primarily developed to suppress the biosynthesis, or to produce insignificant levels, of harmful alkaloids, usually ergovaline and/or lolitrem b, changes in animal respiration may indicate intoxication. The consumption of SE+ forage did not significantly affect animal respiration compared with consuming E+ forage, but respiration was higher than in animals consuming ME– forage (Figure 1B; Table S1A,B in the supplementary material online). A striking pattern appears for the tall fescue cultivar ‘Himag’, for which the high toxicity caused by its natural wild endophyte (SE+ versus E+, cultivar:  $Q_b = 3.000$ ,  $P < 0.465$ ,  $df = 1$ ) was not significantly reduced by the selected strain ‘HiMag4’ (SE+ versus ME–, cultivar:  $Q_b = 0.111$ ,  $P = 0.738$ ,  $df = 1$ ) but by removing the endophyte (ME– versus E+, cultivar:  $Q_b = 11.980$ ,  $P < 0.001$ ,  $df = 1$ ). However, these results may be highly biased, suggesting that more studies are needed (Table S2A–C in the supplementary material online). The lack of clear-cut differences in animal respiration between SE+ and E+ may be because there are unknown effects of any of the other groups of alkaloids (peramine and lolines) or metabolites other than the target alkaloids [45,46].

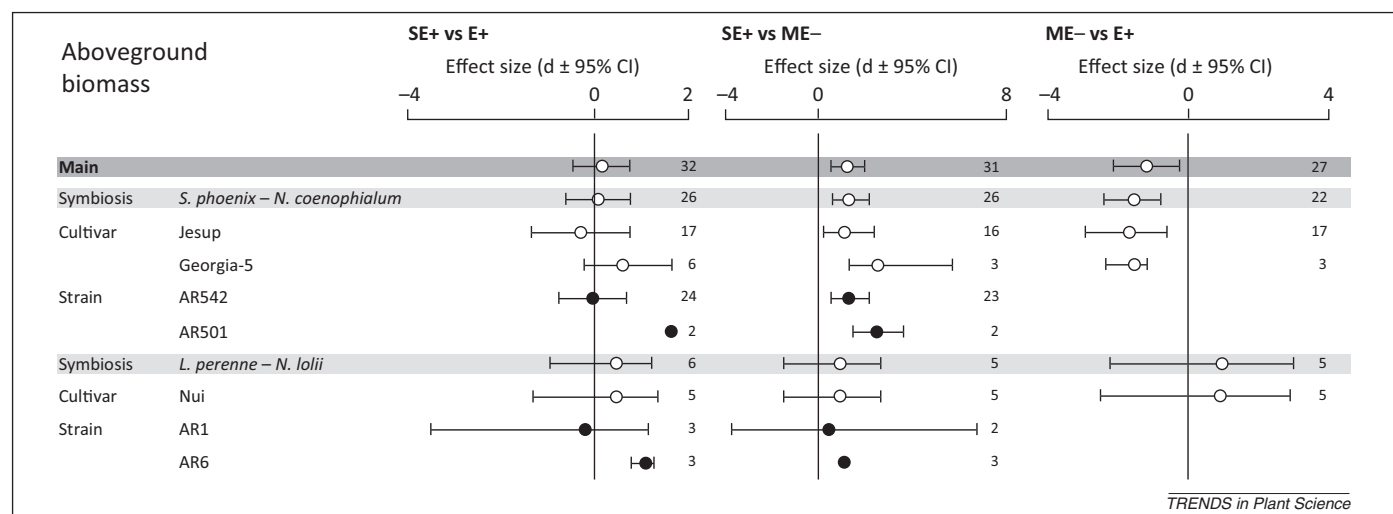
### Maximizing the yield production

In addition to avoiding animal disorders caused by the ingestion of alkaloids, the primary breeding aims have been to improve forage yield by introducing fungal strains that increase seedling establishment, plant growth, seed production, and tolerance of stresses, such as drought, low temperatures, and pest and pathogen attacks [31,33]. Overall, the yield production (aboveground biomass) of SE+ host plants was equal to that of E+ plants, and was greater than that obtained from ME– plants (Figure 2). In this last comparison (SE+ versus ME–), there seemed to be differences in growth between the two grass species (tall fescue and perennial ryegrass); however, the differences remained statistically insignificant and the reliability of the tests was low because of the low number of individual cases ( $Q_b = 0.176$ ,  $P < 0.717$ ,  $df = 1$ ). By contrast, the impaired plant growth caused by removing the natural wild endophyte (E+ versus ME–), appeared to be clear in tall fescue but not in perennial ryegrass (tall fescue versus perennial ryegrass:  $Q_b = 7.610$ ,  $P = 0.005$ ,  $df = 1$ ) (Figure 2). Unfortunately, the very small number of studies is not sufficient to run statistical analyses on the tillering or root biomass of plants (Table S2A–C in the supplementary material online), despite the fact that these two variables are important for grazing and drought tolerance.

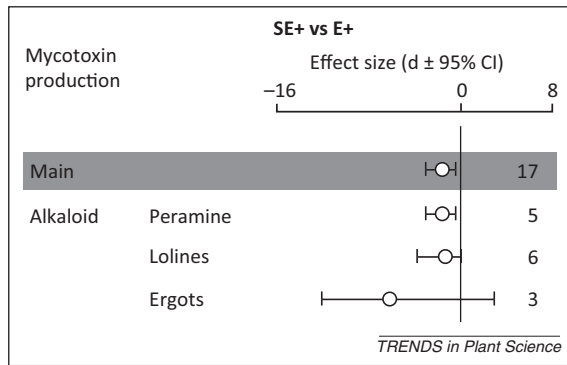
### Mycotoxin production, pest management, and plant community consequences

Recent evidence indicates that the direct antiherbivore properties of endophytes may be exploited for use in biocontrol through developing natural pesticides or improving pest resistance in grass cultivars [5,25,28,31,32]. In addition to the economic benefits derived from using endophytes as a natural biocontrol owing to the reduced need for investment in chemical pest control, consumers would benefit from a reduction in chemical pesticide residues in crops, meat, and milk.

Although not statically robust [main of total alkaloids:  $49.9 > 5 \cdot (14) + 10$ ], our meta-analysis showed a clear



**Figure 2.** The effects of novel associations between host grasses and fungal endophytes on host grass performance. Mean effect sizes (d) and confidence intervals (CI) of the comparisons between SE+ and E+ plants, SE+ and ME– plants, and ME– and E+ plants to show the effects of selected endophytes on aboveground biomass. Within each species-specific symbiotic association (symbiosis), white symbols represent cultivars and black symbols represent fungal strains. The number of studies is indicated to the right of each value.



**Figure 3.** The effects of novel associations between host grasses and fungal endophytes on mycotoxin production. Mean effect sizes (d) and confidence intervals (CI) of the comparisons between SE+ and E+ plants to show the effects of a novel association between host grasses and selected endophytes on total alkaloids production (peramine, lolines, and ergots) and on each group of alkaloids, in the host grass. Peramine and lolines are known to deter some insect pests, whereas ergots cause toxicosis in livestock. The number of studies is indicated to the right of each value.

tendency for SE+ plants to produce lower level of total alkaloids (peramine + lolines + ergots) compared with E+ plants (Figure 3). The same pattern of results is also found when the peramine and loline alkaloids are analyzed independently, even with lower statistical support (Table S2A in the supplementary material online). The very low number of reports on lolitrem b did not allow for any statistical test (data not shown). Besides the low number of articles, the undetected difference between SE+ and E+ in ergot production might be because the endophyte in perennial ryegrass (*N. lolii*) is selected for low production of lolitrem b, although it can still produce some level of ergots (e.g., [45]). In addition, even though endophytes in tall fescue are selected for producing insignificant amounts of ergot alkaloids, some SE+ plants appear to be able to produce traces of these compounds (e.g., [47]).

Neither below- nor aboveground herbivory were affected by the selected endophytes when compared with E+ plants (aboveground herbivores:  $n = 18$ , effect size = 0.520, 95% CI = -0.243, 1.344; belowground herbivores:  $n = 11$ , effect size = -0.592, 95% CI = -1.200, 1.127) or E- plants (aboveground herbivores:  $n = 19$ , effect size = 0.495, 95% CI = -0.104, 1.129; belowground herbivores:  $n = 11$ , effect size = -0.367, 95% CI = -0.668, 0.208). There were not enough reports to estimate the effect of selected endophytes at the level of symbiosis. Within the symbiosis between *S. phoenix* and *N. coenophialum*, aboveground herbivory depended on the cultivar considered ( $Q_b = 21.854$ ,  $P < 0.001$ ,  $df = 1$ ) and was higher on SE+ than on E+ in cultivar 'Georgia-5' but not in cultivar 'Jesup' (Figure 4A). Similarly, only in 'Georgia-5' did the SE+ (inoculated with 'AR542') plants showed a reduced and increased aboveground herbivory compared with ME- plants ( $Q_b = 46.729$ ,  $P < 0.001$ ,  $df = 1$ ) and E+ plants ( $Q_b = 61.385$ ,  $P < 0.001$ ,  $df = 1$ ), respectively. However, all these comparisons are based only on few articles (Table S2A–C in the supplementary material online). Belowground herbivory for the comparison between SE+ and E+ did not differ between symbioses or cultivars (Table S1A in the supplementary material online). However, selected endophyte strains differently affected

belowground herbivores ( $Q_b = 12.142$ ,  $P = 0.006$ ,  $df = 3$ ). Interestingly, perennial ryegrass plants infected with selected fungal strains AR17 and AR37 appeared to be more resistant to belowground herbivores than were either E+ or ME- plants (Figure 4B).

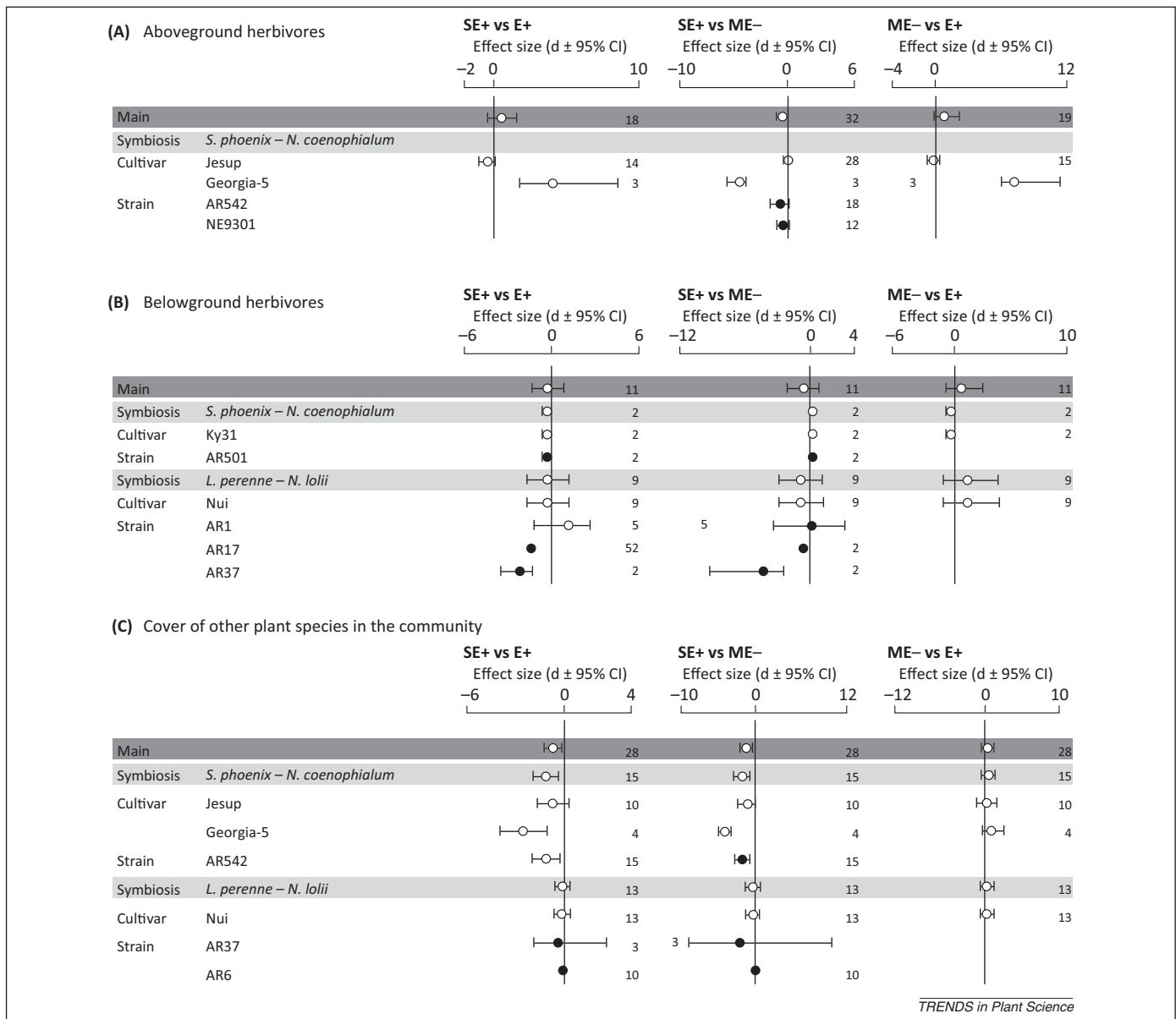
These results suggest that the feasibility of using SE+ plants in forage pest management varies among fungal lineages, their alkaloid profiles as well as potential metabolites other than alkaloids [46], and on the susceptibility of herbivore species to them. For example, the meadow fescue (*Lolium pratense*) cultivar 'Kasper', which was naturally infected by the endophyte *Neotyphodium uncinatum*, was shown to affect negatively field voles (*Microtus agrestis*) [48], whereas closely related sibling voles (*Microtus levis*) were observed to perform equally well on both E+ and E- plants of the same cultivar [49]. However, both studies indicated that the consumption of an E+ diet may have variable effects on higher trophic levels in communities [50].

SE+ plants have a negative effect on other plant species in the plant community compared with E+ (SE+ versus E+:  $n = 28$ , effect size = -0.747, 95% CI = -1.217, -0.313) and with ME- plants (SE+ versus ME-:  $n = 28$ , effect size = -0.772, 95% CI = -1.4455, -0.2076) (Figure 4C). This significant negative effect was stronger on tall fescue plants ( $Q_b = 4.028$ ,  $P = 0.045$ ,  $df = 2$ ) and depended on the cultivar ( $Q_b = 9.844$ ,  $P = 0.007$ ,  $df = 2$ ). Again, the cultivar 'Georgia-5' showed the stronger effects (Figure 4C). This supports the general idea that endophytes can mediate the adaptive radiation, invasion, and competitive success of their host plants in successional plant communities [21,37,39] and that, in agriculture, these plant symbionts can prevent weed invasions [40]. Similar to the situation with GMOs, the question is: what are the long-term consequences of introducing these heritable SMOs to ecosystems?

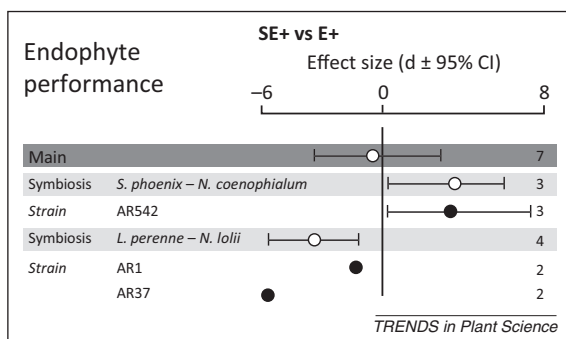
### Stability of human-made grass–endophyte symbiots

Accumulating evidence has revealed that several selective forces can destabilize the grass–endophyte symbiosis [15] and, thus, lead to the loss of the endophyte infection and changes in the phenotypic traits of the cultivar [20,51]. Grass–endophyte symbioses appear to be highly specialized and specific at both the host-species and -genotype level [15,52]. However, the stability and persistence of the symbiosis appears to be dependent on both the fungus and the host grass genotypes and their genetic match [15,53] (but see [54,55]), and to be particularly pronounced in some cultivars of agricultural grass species in high-nutrient environments [16,20,24,40,54]. Given that a lack of nutrients rarely destabilizes the symbiosis in agroecosystems, we propose that genetic mismatch is more likely to constrain novel combinations of fungi and host grass in agricultural environments [24,54].

Our meta-analysis suggests that endophyte performance did not differ between SE+ or E+ endophytes, either when measured as fungal biomass in the plant or as the frequency dynamics of infected plants in population (SE+ versus E+:  $n = 7$ , effect size = -0.479, 95% CI = -3.398, 2.781) (Figure 5). However, there was a significant heterogeneity between hosts ( $Q_b = 12.657$ ,  $P < 0.001$ ,  $df = 1$ ), showing that even when SE+ is not a significant effect



**Figure 4.** The effects of novel associations between host grasses and fungal endophytes on invertebrate herbivores and other species of the plant community. Mean effect sizes (d) and confidence intervals (CI) of the comparisons between SE+ and E+ plants, SE+ and ME– plants, and ME– and E+ plants to show the effects of novel associations between host grass and selected endophytes on (A) aboveground herbivores, (B) belowground herbivores, and (C) the cover of other species in the plant community. Within each species-specific symbiotic association (symbiosis), white symbols represent cultivars and black symbols represent fungal strains. The number of studies is indicated beside each value.



**Figure 5.** The performance of selected endophytes in novel associations with host grasses. Mean effect sizes (d) and confidence intervals (CI) of the comparisons between SE+ and E+ plants to show the endophyte growth in the plant and the infection frequency in populations under the novel associations. White symbols indicate the total main effect and the main effect for each symbiosis, while black symbols represent fungal strains.

at the symbiosis level (SE+ versus E+:  $n = 7$ , effect size =  $-0.5365$ , 95% CI =  $-3.395, 2.673$ ), there is an opposite effect of SE+ in tall fescue and perennial ryegrass. However, given that variation has been found in terms of symbiosis dynamics under real production conditions and is dependent on both host genotype and strain [53,56–58], there is an urgent need for more studies testing the stability of these novel associations.

### Concluding remarks

Our meta-analysis of the literature suggests that the forage quality and sustainability of grass productivity can be enhanced by using endophytes that are SMOs. Fungal strains that do not produce the mycotoxins targeted to livestock, but are capable of maintaining resistance to plagues, enhancing seedling establishment, plant

growth, and stress tolerance (e.g., to stresses such as drought and low temperatures) can be used to improve forage persistence and productivity when introduced into cultivars. These direct antiherbivore properties of endophytes may be exploited in biocontrol, through improvement of plague, pathogen and weed resistance in SE+ cultivars. In addition to economic benefits, through lower investment in agrochemical controls when using natural biocontrols, consumers will be exposed to lower levels of pesticide residues in the crop. However, the low number of studies with few model systems fails to capture the breadth of genetic variability in wild grass–endophyte symbiosis, restricting the strong conclusions of their potential in agriculture. If the genetic potential of fungi limits their applicability, the next step would be to use GM fungal strains in human-made plant–fungus associations (e.g., [32]). Similar to the situation with GMOs, a better understanding of the variability and complexity of the use of SMOs is needed to understand and predict fully their long-term effects in ecosystems. For example, the selected fungal endophyte may increase the invasiveness and competitiveness of its host species, thus threatening the native biodiversity in an invaded ecosystem [37,59].

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#### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.tplants.2013.03.003](https://doi.org/10.1016/j.tplants.2013.03.003).

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<https://secure.faseb.org/FASEB/meetings/summrconf/Programs/11638.pdf>

### 7th EPSO Conference

1–4 September, 2013

Porto Heli, Greece

<http://www.epsoweb.org/7th-epsa-conference-1-4-september-2013-greece>

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