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Epichloë grass endophytes in sustainable agriculture

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There is an urgent need to create new solutions for sustainable agricultural practices that circumvent the heavy use of fertilizers and pesticides and increase the resilience of agricultural systems to environmental change. Beneficial microbial symbionts of plants are expected to play an important role in integrated pest management schemes over the coming decades. *Epichloë* endophytes, symbiotic fungi of many grass species, can protect plants against several stressors, and could therefore help to increase the productivity of forage grasses and the hardiness of turf grasses while reducing the use of synthetic pesticides. Indeed, *Epichloë* endophytes have successfully been developed and commercialized for agricultural use in the USA, Australia and New Zealand. Many of the host grass species originate from Europe, which is a biodiversity hotspot for both grasses and endophytes. However, intentional use of endophyte-enhanced grasses in Europe is virtually non-existent. We suggest that the diversity of European *Epichloë* endophytes and their host grasses should be exploited for the development of sustainable agricultural, horticultural and landscaping practices, and potentially for bioremediation and bioenergy purposes, and for environmental improvement.

Global agriculture faces major challenges, two of them being the heavy use of synthetic pesticides and the changing environment brought about by climate change[1,2]. Microbial symbionts of crop plants could provide solutions for mitigating these problems by improving plant fitness and resistance to several stressors. Grasslands in particular are under pressure, as they represent 70% of the world's agricultural area[3,4], with approximately 58% and 70% of dairy- and meat-based dietary protein, respectively, being derived from grasslands[5].

Today, the productivity of intensive farming relies heavily on the use of chemical pesticides[6,7]. However, increased global concern about the impacts of synthetic plant protection substances on human and animal health is driving changes in agricultural production practices and related legislation[8]. For example, the European Union (EU) promotes use of less harmful chemicals in agriculture through the European Pesticide Regulation (EC) No. 1107/2009. The substitution principle, that is, the use of the least hazardous alternatives, determined through comparative risk assessments of plant protection products, will be applied in the EU[9], and stricter legislation has been implemented to ensure tighter control of pesticides in other parts of the world[10]. Tighter regulation of agrichemicals, in turn, poses challenges to plant production, as it can lead to reductions in crop yields. Consequently, there is a need for alternative pest control methods, such as integrated pest management — a system-wide approach that minimizes the use of pesticides[2,7]. New synthetic solutions, such as the use of genetically modified organisms (GMO), are not being put to use globally because of concern about their effect on health and the environment. The EU has probably the strictest regulations in the world for GM products[11], but tight regulations on GMOs are also applied elsewhere, for example in Japan and New Zealand[12]. Instead of (or in conjunction with) man-made technologies, we could take advantage of naturally occurring agents to make agriculture more sustainable and environmentally friendly.

In addition to the overuse of synthetic pesticides, climate change also sets challenges for today's agriculture. Extreme weather conditions are expected to become more frequent, and increasing water scarcity, together with overgrazing and erosion, are threatening many agricultural regions, especially dry grasslands[1,13]. Although climate change may expand the extent of arable land area, there may also be a concomitant spread of new pests, pathogens and weed species[1]. Thus, there is a need to increase drought resistance of both forage and crop plants, and to develop new plant protection methods and practices to enhance the resilience of agriculture to the potential effects of climate change.

Epichloë endophytes can contribute to the establishment of sustainable agricultural systems[14,15], as they are natural fungal symbionts of many agriculturally important grass species and can improve the fitness of their hosts[16]. It is also possible to create new grass cultivars by inoculating grasses with endophytes. Compared with other plant-fitness-promoting microbes that can be used in agriculture and need to be continuously added to the production environment (such as mycorrhizal fungi[17] and rhizobacteria[18]), systemic *Epichloë* endophytes are maternally inherited and persist in grass lines after inoculation[19,20]. The ability of these endophytes to improve agricultural and turf production is well acknowledged and taken into account in grass breeding, especially in the USA, Australia and New Zealand. In Europe, however, endophytes are largely ignored in grass breeding programs and in agriculture, even though they have been documented in many European cultivars[21,22]. Detailed reviews of plant breeding, development and the success of *Epichloë* endophytes are provided elsewhere[14,23,24,25].

Biology of *Epichloë* endophytes

Epichloë endophytes are one of the most studied genera of the ascomycete family Clavicipitaceae, and in endophyte research in general[26,27]. They are symbiotic fungi that inhabit above-ground plant tissues without causing apparent disease symptoms[16]. *Epichloë*

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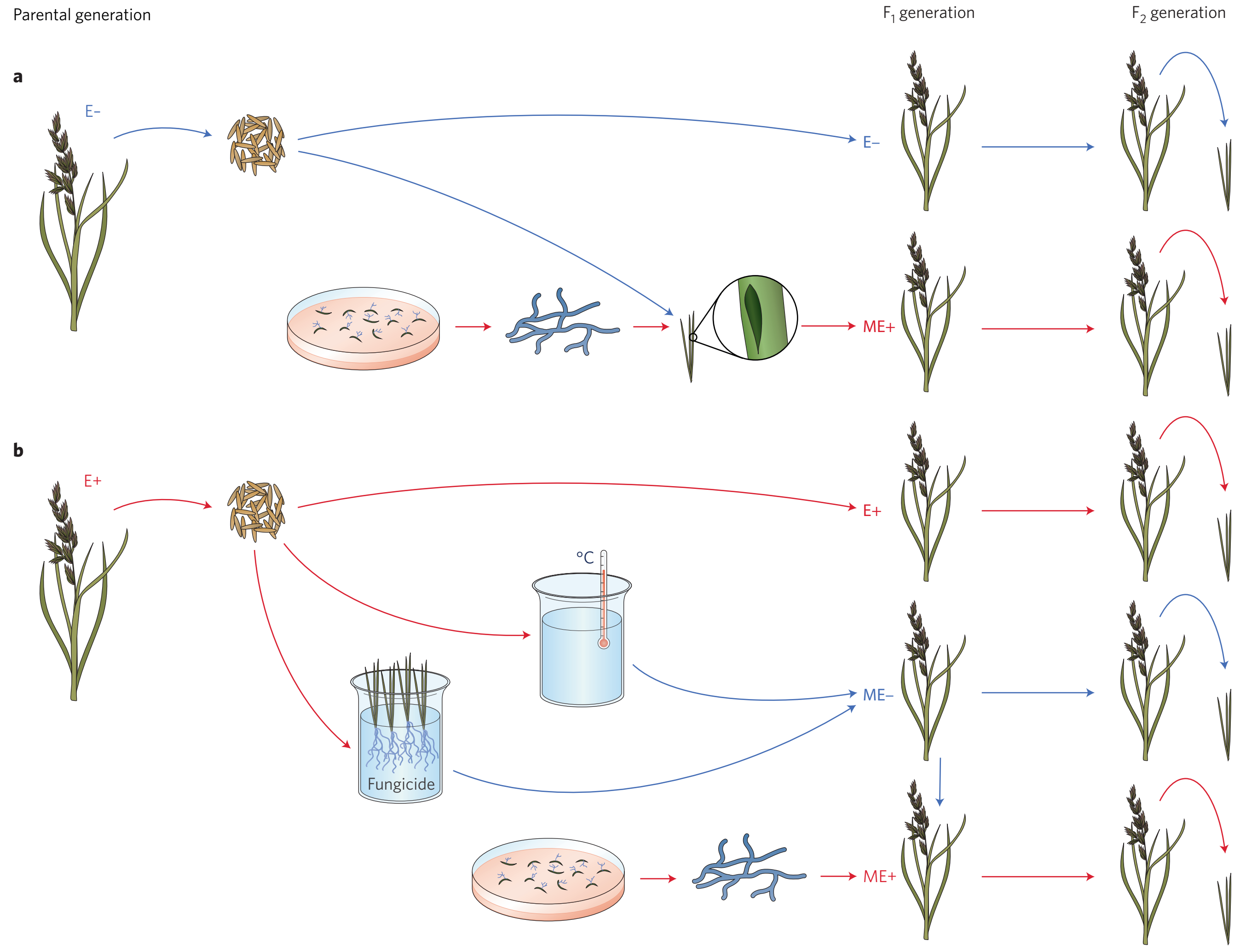

Figure 1 | Vertical transmission of *Epichloë* endophytes in grasses and manipulation of endophyte infection. *Epichloë* endophytes are systemic fungal endophytes that are transmitted vertically to the next plant generation via grass seeds. **a**, Infection of *Epichloë* endophytes can be manipulated to produce endophytic grass lineages. To achieve this, grass seedlings are inoculated with fungal hyphae through a small longitudinal cut above the meristematic region. Adult grass tillers can be inoculated by placing fungal hyphae into a cut at the leaf base[24,98,99]. **b**, Endophyte-free grass lineages can also be produced. To do this, endophytic grass seeds are heat-treated or grass tillers are treated with certain fungicides[98]. Grasses: E+, endophytic; E−, endophyte-free; ME+, manipulatively endophytic; and ME−, manipulatively endophyte-free.

endophytes are found almost exclusively in grasses from the subfamily Pooideae, and many species tend to be host specific. They grow intercellularly and systematically, infecting both vegetative and reproductive organs of their host plants. The fungus grows inside plant tissues including inflorescences and developing seeds, and is carried over to the next grass generation via vertical transmission (Fig. 1). Some *Epichloë* species are capable of sexual reproduction, where the fungus produces fruiting bodies on the inflorescences and is then transmitted horizontally via spores[16,24,27]. Theoretically, horizontal transmission may also be possible, via asexual conidia produced on the leaf surface[28].

Epichloë endophytes have complex symbiotic interactions with grasses. They can defend their host — their source of photosynthetic products and nutrients — against herbivores by producing toxic compounds[29]. They can also benefit their hosts by enhancing plant growth, photosynthetic rate and tolerance to biotic and abiotic stresses[30,31,32]. Endophytes, on the other hand, receive protection, nutrients and reproduction mechanisms (via vertical transmission) from their hosts[33]. Because they increase plant fitness and infect many of the pooid grass species, including some important agricultural species[33,34], these fungi are noteworthy candidates for agricultural uses[27,35]. Interest in sustainable and renewable agricultural production increases constantly, and *Epichloë* endophytes may offer an environmentally friendly method to increase crop productivity and hardiness of turf grasses while reducing usage of chemical fertilizers, pesticides and fungicides[36]. Due to endophyte-promoted resistance to environmental stresses such as drought, endophytes could also prove to be beneficial in grass-dominated communities in a changing climate[37].

Current uses

Development and commercialization of *Epichloë* strains for agricultural uses began when endophyte-produced bio-protective alkaloids were identified in the 1980s, which led to the search for endophytic grasses that were resistant to pest herbivory, yet safe for livestock[23]. Since then, the advantages that selected endophytes bring to the fitness and productivity of grasses have been well documented[38,39]. Selected endophytes can improve plant growth and resistance to pests and pathogens (summarized in Table 1). These effects lead to increased persistence and yield in pasture grasses.

Table 1 | Current and potential uses of *Epichloë* endophytes.

Examples of the current usage of endophytes	Benefit provided by endophyte	Endophyte	Host species	Where?	Refs
Agriculture	Improved plant growth	*Epichloë coenophiala*	*Schedonorus phoenix*	Pasturelands, USA	85
		Epichloë festucae var. *lolii*	*Lolium perenne*	Pasturelands, New Zealand and Australia	86,87
Agriculture	Improved drought tolerance	*Epichloë coenophiala*	*Schedonorus phoenix*	Pasturelands, USA	88
		Epichloë festucae var. *lolii*	*Lolium perenne*	Pasturelands, New Zealand and Australia	89
Agriculture	Improved pest resistance	*Epichloë coenophiala*	*Schedonorus phoenix*	Pasturelands in USA, limited use in New Zealand and Australia	23,24
		Epichloë festucae var. *lolii*	*Lolium perenne*	Pasturelands in New Zealand and Australia, limited use in USA	23,24
Bird and wildlife deterrent	Improved protection against herbivores	*Epichloë coenophiala*	*Schedonorus phoenix*	Tall fescue pastures, airports, recreational areas, near gardens	42,44
		Epichloë festucae var. *lolii*	*Lolium perenne*	Ryegrass recreational areas, near gardens	42,44
Potential uses					
Pathogen protection	Improved pest protection				46
Weed protection	Improved competitive abilities				31
Prevention of overgrazing	Improved protection against herbivores				
Prevention of soil erosion	Improved plant growth				
Restoration of native/semi-native grasslands	Improved plant growth				33
Establishment of crops in new, presently unsuitable, areas	Increased adaptive potential, plant fitness				36
Remediation of contaminated soils	Removal of toxic compounds from soil				53
Bioenergy production	Improved plant growth				52
Mitigation of climate change	Increased adaptive potential				37
	Increased carbon sequestration				59

As a result, many farmers use endophyte-enhanced grasses, especially in the USA, New Zealand and Australia[39]. MaxQ is an example of a commercial endophytic strain (*Epichloë coenophiala)* that infects the tall fescue variety Jesup, and is currently marketed in the USA[38,40]. MaxQ provides markedly better stand survival as well as higher livestock productivity and net returns per cow compared to endophyte-free tall fescue[40]. In ryegrass, novel endophytes (such as AR1, AR5, AR37 and NEA2) are marketed in New Zealand and Australia, and possess effective insect-deterrence capacities[23,25]. The adoption of novel endophytes has been successful[25], especially in New Zealand, and today selected endophyte strains contribute approximately NZ$200 million annually to the country's economy; indeed, the value of the commercial endophyte strain AR37 (*Epichloë festucae* var. *lolii*) alone was estimated to be NZ$42 million during the period 2007–2011[23]. AR37 provides excellent pest resistance coupled with higher herbage production and persistence to ryegrasses[25].

Epichloë endophytes have various practical applications beyond agriculture — potentially anywhere where grass herbivory is unwanted, or herbivory effects have no importance. For example, turf grass cultivars containing selected endophytes have been commercialized[41] and are marketed as sustainable, pest-deterring and visually appealing. Endophytic grass cultivars are available, for example, for use on golf courses, recreational areas and private lawns. Beneficial endophytes are also used as wildlife-deterring agents in *Lolium* and *Festuca* grasses; some *Epichloë coenophiala* and *Epichloë festucae* var. *lolii* strains are effective bird deterrents and have been patented[42]. These endophytic grass cultivars can be planted in airports, sports fields and recreational areas, or near food crop fields, to effectively deter birds or other herbivorous wildlife[42–44].

Untapped potential

Because of their ability to increase plant growth, stress tolerance and overall fitness, *Epichloë* endophytes could have applications in a number of other fields. Given their ability to increase plant drought tolerance, endophytes could be used to widen or maintain the cultivation areas of specific forage and pasture grasses, and could therefore contribute to sustainable rural development[32,36]. And as the presence of endophytes in grasses can decrease weed biomass, *Epichloë* endophytes could be used against weeds. On meadow fescue fields, for instance, endophyte infection reduces both weed biomass and the number of weed species[31]. Such use of endophytes in turn could lead to a reduction in the use of synthetic herbicides, such as glyphosate. Additionally, *Epichloë* endophytes have potential for use against pathogens in agricultural and horticultural settings,

Table 2 | Endophytic grass cultivars used in Europe.

Grass species	Cultivar	Endophyte infection frequency (%)	Country of origin	Refs
Perennial ryegrass (*Lolium perenne*)	Argona	6	Poland	90
	Nadmorski	5	Poland	90
	Solen	4	Poland	90
	Wiecławicki	4	Poland	90
	Norlea	7–9	Canada/Finland	91
	Riikka	1	Finland	91
	Barlenna	20	Holland	92
	Gremie	15	Holland	92
	S23	5	Wales	92
	S24	20	Wales	92
Meadow fescue (*Schedonorus pratensis*)	Antti	10	Finland	91,22
	Boris	1–100	Finland/Sweden	91,93
	Kalevi	5–74	Sweden/Finland	91,22
	Kasper	0–96	Sweden/Finland	91,79,22
	Salten	96–100	Norway/Finland	91,22,93
	Minto	22	Sweden	94
	Norild	60–100	Norway/Sweden	79,94
	Inkeri	90–100	Finland	79,22
	Ilmari	<10	Finland	22
	Bottina	14	Sweden	93
	Leto	86	Denmark	93
	S215	60	Wales	92
	Bf855	85	Wales	92
	Bf998	15	Wales	92
	3 endophytic cultivars	45–65	Poland	95
	14 endophytic varieties	2–95	Germany	96
Tall fescue (*Schedonorus phoenix*)	Wrangler	<10	Central Europe	97
	Dovey	10	Wales	92
	6 endophytic varieties	4–54	Germany	98
Red fescue (*Festuca rubra*)	10 endophytic varieties	1–73	Germany	96
Sheep fescue (*Festuca ovina*)	2 endophytic varieties	16–78	Germany	96

Number of endophyte-free cultivars detected: perennial ryegrass, 28; Italian ryegrass *Lolium multiflorum*, 11; meadow fescue, 27; tall fescue, 43; red fescue, 100; and sheep fescue, 16.

as they have been demonstrated to inhibit a range of fungal pathogens[45]. In fine fescue cultivars, endophyte infection has been shown to reduce infection of dollar spot in field conditions[46], and there are indications that endophytes can suppress disease incidences in other grasses as well[47–49]. Furthermore, endophyte-infected grass cultivars could be sown next to crops to act as a buffer against some diseases, herbivores and weeds, and could therefore help to reduce crop yield losses[31,49,50]. Synthetic symbioses between *Epichloë* endophytes and important cereal crops are under development[51].

Improved biomass production conferred by *Epichloë* endophytes in grasses may also benefit bioenergy production[52]. In fact, even the most toxic endophyte strains could be used for production of bioenergy crops, which are not used for animal feed. This application could be the most feasible use of endophytes, especially in areas where intense agriculture is not profitable (such as in cold or arid conditions). Furthermore, production of endophytic plants for energy use would be cost effective. Set-aside fields would be cheap to establish and require very low maintenance, but would still have great production potential because of endophyte-promoted yield without chemical pesticides. Another important application could be the remediation of contaminated soils, as endophytes can remove total petroleum hydrocarbons and polycyclic aromatic hydrocarbons from oil-contaminated soils[53] and can improve plant tolerance to toxic heavy metals, such as zinc[54]. Besides these biotechnological applications, endophytic grasses could be used for conservation purposes[55], to prevent overgrazing or soil erosion, or to restore native or semi-native grasslands[33]. Endophytes may even alleviate problems brought on by climate[33,37]. Elevated CO_2 concentrations increase endophyte infection frequencies[56], plant biomass, and the carbohydrate and protein content of endophytic plants[57,58]. Warming and drought stress have been demonstrated to alter alkaloid production in endophytes[56], and increase the growth[37,59] of endophyte-infected plants compared to controls. Recent evidence suggests that *Epichloë* endophytes can contribute to soil carbon sequestration, as they can increase soil carbon pools in tall fescue pastures[60,61], which could be beneficial in efforts to mitigate global warming.

Potential problems

Taking full advantage of the potential of *Epichloë*-endophyte-improved grass cultivars requires an acknowledgement of the costs and potential risks associated with endophyte-grass interactions. Some endophytes can cause harmful impacts on grazing mammals, a condition known as livestock toxicosis. Symptoms include intense neuromuscular symptoms and reduced reproduction, weight gain and milk production, as well as tissue inflammation, which can lead to necrosis of body extremities[23]. Livestock toxicosis has been a serious problem in countries where endophytic forage grasses are used; in the US, it has led to estimated losses of over US$1 billion annually[62]. Thus, the possibility of toxicosis incidences should be seriously considered when using endophytes in forage grass improvement. Advances in our understanding of the genetic variation in alkaloid biosynthesis in *Epichloë* endophytes, and the genes encoding key steps of the biosynthesis pathway, have enabled the development of safe strains[63]. Livestock toxicosis could then be avoided without losing the benefits of the symbiosis by applying endophyte–grass combinations that are safe for livestock but that promote yield and resistance to pests and pathogens[14,33,64]. Indeed, such novel endophyte–host-grass combinations have already been developed and successfully commercialized, for example in New Zealand[23,25]. However, the successful use of endophytes in forage production requires a thorough understanding of the variation in chemical profiles and concentrations of toxic compounds of endophyte–host-grass combinations in different environments[14,63].

In addition, endophyte infections can be unstable, because the fungus can lose its viability in the heritable mother plant lineage[65]. Endophyte infection can be lost from grass seeds because of improper storing[66] or imperfect vertical transmission[67]. At least in theory, grasses could also become infected with wild endophyte strains, or the inoculated endophyte may hybridize with wild endophyte strains[27], which could alter the properties of endophytic grass cultivars. Therefore, continuous monitoring of the endophyte status and endophyte genotype of commercial cultivars is needed to ensure stability of the endophyte–grass combination. The methodology for maintaining endophyte strains in grass lineages should also be studied and developed further.

Finally, endophyte-induced plant responses are functions of two-way interactions with the environment. The pheno- and chemotype of the symbiotum is dependent on abiotic and biotic environmental factors[30,68–71]. The effects of endophyte-induced plant responses on other species in the community seem to be context dependent. In particular, reports on endophyte effects on pathogens are variable and partly controversial[35,72,73]. Animal responses to endophytes can also vary depending on the animal and grass species, endophyte strain and environmental conditions[25,72]. Furthermore, cascading effects of endophytes on other trophic levels have been reported[74]. Consequently, introduced endophytes are likely to have unpredictable and random effects on local communities of the plant host and environment. By increasing competitive ability of the host grass, endophytes could prevent invasions of other plants[31,75] and reduce local biodiversity. In extreme cases, fungal endophytes have been shown to increase the invasiveness of host grasses and thus threaten the biodiversity of the native ecosystem[21,33]. Therefore, grass–endophyte combinations should be tested separately for each purpose and target habitat. We propose that native and local endophyte–grass combinations should be preferred as a source for cultivar improvement programmes, and suggest that the spread of alien invasive endophytes and grasses to natural habitats should be eschewed to avoid the risk of biological hazards.

European potential

The use of *Epichloë* endophytes in agriculture in Europe is yet to take off, even though many European grass cultivars possess great potential for improvement through endophyte infection. *Epichloë* endophytes are natural symbionts of several wild grasses in Europe[21,76,77]. However, most European forage grass cultivars are endophyte free or their endophyte infection levels are low, and endophytes are not intentionally used to improve grass cultivars. However, some European meadow fescue (*Schedonorus phoenix*), red fescue (*Festuca rubra*) and sheep fescue (*F. ovina*) cultivars have infection frequencies of more than 50%, although infection of cultivars of perennial ryegrass (*Lolium perenne*) is mostly less than 10%[21,22,78,79] (Table 2). And some European meadow fescue cultivars are reported to have high endophyte infection frequencies by strains that improve host biomass production in different agricultural settings[31,69,71]. In some cases endophyte infection has been shown to double the yield of meadow fescue[31]. Because alkaloids produced by these endophytes are mainly toxic to insects[50], these strains have great value for agriculture, as meadow fescue is an important forage grass in Europe.

Low use of endophytic cultivars in Europe may partly be a consequence of some European countries imposing limits on endophyte infection levels in commercial grass cultivars to avoid potential livestock toxicosis[80]. In addition to the lack of intentional use of endophytic forage and turf grass cultivars, there is a tradition to use botanically diverse, polyculture pastures in Europe. These agricultural management strategies reduce the possibilities for serious endophyte toxicosis compared to the USA, New Zealand and Australia, where monoculture pastures are more prominent. These factors might explain why *Epichloë* endophytes have not received so much attention in European agriculture[80]. The breeding potential in Europe, however, is especially high, because many of the important endophytic forage grasses originate from Europe[21,81] and the biodiversity hotspots of both grasses and their endophytes occur in Europe and the Middle East[80,82]. In fact, European grass populations serve as a reserve for novel endophyte and grass strains for breeding of forage grasses worldwide[83,84].

Outlook for endophyte exploitation

We propose that *Epichloë* endophytes should be considered when developing sustainable management strategies for agriculture, especially in Europe, where there are biodiversity hotspots of these organisms. Endophyte-enhanced grasses could be used as alternatives for synthetic plant protection products, either by themselves, or integrated with traditional products. Finding and developing new endophyte–grass combinations may open up the possibility of using endophytes in completely new practices, such as for the breeding of cereal cultivars, bioenergy, bioremediation, and environmental improvement and restoration. As such, more attention and resources should be invested in researching and developing *Epichloë* endophyte applications.

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Additional information

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Competing interests

The authors declare no competing financial interests.