Received: 17 November 2023 | Revised: 19 March 2024 | Accepted: 29 March 2024

DOI: 10.1111/pce.14912

ORIGINAL ARTICLE

Plant, Cell & Environment WILEY

The growth promotion in endophyte symbiotic plants does not penalise the resistance to herbivores and bacterial microbiota

Wei Zhang[1] | **Pedro E. Gundel**[2] | **Ruy Jáuregui**[3] | **Stuart D. Card**[1] | **Wade J. Mace**[1] | **Richard D. Johnson**[1] | **Daniel A. Bastías**[1]

[1]AgResearch Limited, Grasslands Research Centre, Palmerston North, New Zealand

[2]Centro de Ecología Integrativa, Instituto de Ciencias Biológicas, Universidad de Talca, Talca, Chile

[3]Animal Health Laboratory, Biosecurity New Zealand, Ministry for Primary Industries, Upper Hutt, New Zealand

Correspondence
Daniel A. Bastías
Email: daniel.bastias@agresearch.co.nz

Funding information
Ministry of Business, Innovation and Employment; Fondo Nacional de Desarrollo Científico y Tecnológico

Abstract

A trade-off between growth and defence against biotic stresses is common in plants. Fungal endophytes of the genus *Epichloë* may relieve this trade-off in their host grasses since they can simultaneously induce plant growth and produce anti-herbivore alkaloids that circumvent the need for host defence. The *Epichloë* ability to decouple the growth-defence trade-off was evaluated by subjecting ryegrass with and without *Epichloë* endophytes to an exogenous treatment with gibberellin (GA) followed by a challenge with *Rhopalosiphum padi* aphids. In agreement with the endophyte-mediated trade-off decoupling hypothesis, the GA-derived promotion of plant growth increased the susceptibility to aphids in endophyte-free plants but did not affect the insect resistance in endophyte-symbiotic plants. In line with the unaltered insect resistance, the GA treatment did not reduce the concentration of *Epichloë*-derived alkaloids. The *Epichloë* mycelial biomass was transiently increased by the GA treatment but at the expense of hyphal integrity. The response of the phyllosphere bacterial microbiota to both GA treatment and *Epichloë* was also evaluated. Only *Epichloë*, and not the GA treatment, altered the composition of the phyllosphere microbiota and the abundance of certain bacterial taxa. Our findings clearly demonstrate that *Epichloë* does indeed relieve the plant growth-defence trade-off.

KEYWORDS
alkaloids, *Epichloë* fungi, growth-defence trade-off, phyllosphere microbes, phytohormones, symbiosis

1 | INTRODUCTION

Plant growth and defence are largely regulated by the action of hormonal signalling pathways (Denancé et al., 2013; Robert-Seilaniantz et al., 2011). Plant growth is controlled by the action of hormones, including gibberellin (GA) and auxins, whilst hormones, including jasmonic acid (JA) and salicylic acid (SA) control defence pathways (Santner et al., 2009). Hormonal signalling pathways, including growth- and defence-related hormonal pathways, are interconnected, and the resulting interactions allow plants to adjust

---

their phenotypes to changing environments (Altmann et al., 2020; He et al., 2022). A trade-off between plant growth and defence against biotic stresses has been commonly observed in plants with enhanced production of chemical defences (Ballaré & Austin, 2019; Lind et al., 2013; Sestari & Campos, 2022; Züst & Agrawal, 2017). This trade-off largely exists due to the mutual inhibition of the hormonal signalling pathways that control plant growth and defences, that is, the induction of GA/auxins generally represses JA/SA responses, as demonstrated in several genetically modified plants (Campos et al., 2016; Jin, Qi, et al., 2023; Li et al., 2019, 2022; Liu et al., 2021; Major et al., 2020; Monson et al., 2022; Panda et al., 2022; Peng et al., 2021). Plant-beneficial microbes that can provide plants with mechanisms of growth promotion and/or protection against attackers may relieve the plant growth-defence trade-off (Bennett et al., 2006; Pangesti et al., 2013). Examples of this are asexual endophytic fungi of the genus *Epichloë* that form mutualistic associations with grasses of the subfamily Pooideae (Johnson et al., 2021; Schardl et al., 2004). These endophytes can simultaneously increase plant defences and enhance the growth of their plant hosts (Bastías et al., 2021; Bastías, Gundel, et al., 2022; Gundel et al., 2013). A meta-analysis of this research topic showed that the *Epichloë*-mediated enhancement of plant resistance did not penalise the growth of their hosts (Bastías et al., 2021). However, manipulative experiments specifically designed to evaluate the *Epichloë*-based alleviation of the plant growth-defence trade-off have yet to be published.

Asexual *Epichloë* spp. colonise the intercellular spaces of aerial host tissues without invading host cells and are vertically transmitted through seed to the next plant generation (Schardl et al., 2004). In leaf blades and sheaths, *Epichloë* hyphae are generally attached to plant cell walls. The growth of *Epichloë* in the elongating leaves is mostly by intercalary hyphal extension in a synchronised manner (i.e., similar extension rate between plant and fungal cells) (Christensen et al., 2008; Scott et al., 2018; Voisey, 2010). Due to the nature of vertical transmission, the fitness of *Epichloë* endophytes is strongly aligned with the fitness of their plant hosts since plant reproduction offers the opportunity for these symbionts to multiply and disperse (Bastías & Gundel, 2023; Gundel et al., 2011). The most documented benefit of *Epichloë* to their plant hosts is the fungal provision of antiherbivore alkaloids (Bastias et al., 2017; Johnson et al., 2021). The *Epichloë*-derived alkaloids are systemically distributed within the aboveground tissues of their plant hosts, with four chemical classes characterised to date: the ergot alkaloids (e.g., ergovaline), indole-diterpenes (e.g., epoxyjanthitrems), pyrrolizidines (e.g., lolines) and pyrrolopyrazines (peramine) (Caradus & Johnson, 2020; Schardl et al., 2004). The effectiveness of *Epichloë*-derived alkaloids against a particular herbivore species depends on the compound and its in planta localisation and concentration (Bastias et al., 2017; Caradus & Johnson, 2020). The plant developmental stage (e.g., vegetative, reproductive), host genotype, availability of water and nutrients (e.g., nitrogen, phosphorus), presence of other microorganisms (e.g., arbuscular mycorrhizal fungi) and herbivore feeding habit regulate the concentration of these compounds in planta (Graff et al., 2020; Hahn et al., 2008; Liu et al., 2011; Rasmussen et al., 2007; Sullivan et al., 2007; Zhang et al., 2009). *Epichloë* can also increase the growth of their plant hosts. Although the exact molecular mechanisms behind this plant growth promotion are still not fully understood, experimental results suggest that these fungi provide and/or induce the host production of growth promoting hormones (Bastías et al., 2021; Gundel et al., 2013). *Epichloë* spp. are well known to produce auxins in culture (De Battista et al., 1990; Xie et al., 2023; Yue et al., 2000). Additionally, when in planta, *Epichloë* spp. can also enhance the plant's production of auxins and stimulate the auxin and GA signalling pathways (Dupont et al., 2015; Liu, Ju, et al., 2022; Mathew et al., 2023; Schmid et al., 2017).

The activation or suppression of phytohormonal signalling pathways can also affect the microbial communities associated with plants (Eichmann et al., 2021). This is particularly true for the defence phytohormones, namely JA and SA, that regulate the presence of microorganisms in plants (Bastías, Balestrini, et al., 2022; Thaler et al., 2012). For instance, the microbiota of *Arabidopsis thaliana* plants was enriched with certain bacterial taxa when the plant's immunity was suppressed via the receptor FERONIA that negatively regulates the JA pathway (Guo et al., 2018; Tang et al., 2022) (see also Pfeilmeier et al., 2021). *Epichloë* spp. can also influence the plant's microbiota, and studies have shown that the diversity of phyllosphere and seed bacterial communities in *Achnatherum inebrians* and *Lolium multiflorum*, respectively, were increased in the presence of *Epichloë* endophytes (Bastías, Bustos, et al., 2022; Liu, Ju, et al., 2022). This effect of *Epichloë* on the plant microbiota may be associated with fungal-mediated changes in the host's chemical composition (e.g., presence of sugars, antioxidants, alkaloids) and/or competition over plant resources (Liu, Ju, et al., 2022; Pérez et al., 2013; Roberts & Lindow, 2014; Zhang et al., 2019). The plant's microbiota may also be shaped by *Epichloë*-mediated changes to the plant's immunity (Bastías et al., 2018, 2019; Liu, Ju, et al., 2022). The in planta levels of the SA analogue 4-hydroxybenzoate were correlated with changes in the abundance of distinct bacterial taxa in the leaves of *Epichloë*-symbiotic plants (Horváth et al., 2007; Liu, Ju, et al., 2022).

To gain an understanding of the ability of *Epichloë* endophytes to relieve the plant growth-defence trade-off, we evaluated the growth and defence responses in plants associated with endophytes that were treated with GA hormone. For this purpose, ryegrass plants with and without *Epichloë* sp. LpTG-3 strain AR37 were subjected to an exogenous application of GA_3 followed by a challenge with *Rhopalosiphum padi* aphids. A promotion of plant growth and a repression of plant defence responses are usually observed after the GA exposition of plants (He et al., 2022). *Epichloë* sp. strain AR37 is well known to produce epoxyjanthitrems, alkaloids that confer strong insect resistance to their plant hosts (Caradus & Johnson, 2020; Finch et al., 2020). We predicted that GA-derived plant growth promotion would reduce the resistance to aphids in *Epichloë*-free plants but would not compromise the insect resistance in *Epichloë*-symbiotic plants due to the fungal production of alkaloids. Furthermore, we also evaluated the microbiota associated with *Epichloë*-free and

Epichloë-symbiotic plants after the GA treatment, predicting that both GA (via suppression of plant defences) and *Epichloë* would affect the composition of the bacterial communities and relative abundances of certain taxa within the ryegrass phyllosphere.

2 | MATERIALS AND METHODS

2.1 | Plant stock and aphid colonies

Seeds from a single population of a long-term tetraploid hybrid ryegrass (*Lolium boucheanum* syn. *Lolium* x *hybridum*) cv. Ohau plants infected with AR37 were sown in small pots within a 12 by 12 tray in early winter and maintained in a glasshouse and watered as required. Two months post-sowing, when the seedlings had approximately three tillers, a single tiller per plant was selected and cut at the base for determining the *Epichloë* status using an immunoblotting technique (Simpson et al., 2012). Twenty endophyte-symbiotic (E+) and 20 endophyte-free plants (E−) of similar size were transferred to 0.4 L pots (16 cm × 6.5 cm) filled with commercial potting mix and maintained in the same glasshouse for 3 months and watered as required. In early spring, a colony of aphids (*R. padi*) were generated from around 100 apterous insects collected from the field. Aphids were placed onto E− plants of the hybrid ryegrass cv. Ohau and maintained for 2 months within a glasshouse.

2.2 | Experimental design and set-up

An experiment was conducted to evaluate the defence response of E+ and E− plants exposed to GA against *R. padi* aphids. Once the selected plants reached 5 months old, they were individually covered with a white and translucent mesh supported by a tubular plastic net that would be used at a later stage to prevent the escape of aphids. Plants were acclimatised under the mesh for 5 days before the application of GA. The entire experiment was carried out in the same glasshouse in which the plants were grown.

The experiment consisted of a 2 × 2 factorial design with *Epichloë* presence (E+, E−) and GA treatment (GA+, GA−) as the main factors. Two days before the GA treatment, plants were trimmed to 10 cm height from the crown to standardise the plant height and potentiate the GA_3 effect as recommended by the hormone manufacturer (Progibb® SG; Nufarm Ltd.). Plant tillers and leaves were counted after trimming. Half of the plants (10 E+ and 10 E−) were sprayed with 20 mL of 0.40 g L^{-1} GA_3 solution, while the other half (10 E+ and 10 E−) were sprayed with 20 mL of tap water. Three days later, 10 aphids from the generated colonies were placed on each plant and then the mesh was used to cover the shoots of each plant. The growth of plants and insect populations were monitored for 4 weeks.

The growth of both plants and insect populations were determined by serially measuring plant height and aphid population sizes (by counting aphids). These measurements were performed at Days 3, 7, 14, 21 and 28 post-GA treatment. At each assessment date, a single tiller per plant was harvested, and a small section of leaf sheath (2–3 mm) was dissected and immersed in 96% ethanol for microscopy examination. These samples were used to evaluate the AR37 hyphal integrity within plant tissues. The remaining tiller material was snap frozen in liquid nitrogen and subsequently freeze-dried. The dried tillers were ground to a fine powder using FastPrep homogeniser (FastPrep®24; MP Biomedicals) and later fractionated for extractions of DNA and metabolites. Total genomic DNA (including plant and *Epichloë* DNA in the case of E+ plants) was extracted from approximately 20 mg of dried and ground tiller using the Nucleospin® Plant II kit (Macherey-Nagel GmbH & Co.) following the manufacturer's instructions. DNA integrity, quality and quantity were determined using 1% agarose gels, a spectrophotometer (Nanodrop®; Thermo Fisher Scientific Inc.) and Qubit 3 Fluorometer dsDNA BR Assay Kit (Invitrogen Corp.), respectively. Metabolites associated with plants were extracted on 50 mg of dry and ground tiller with 1 mL of water-acetone (1:4) following the method described in Hennessy et al. (2016). The extracted DNA was used to quantify the in planta *Epichloë* mycelial biomass through real-time quantitative PCR (qPCR) and to characterise the phyllosphere bacterial microbiota through amplicon sequencing. The extracted metabolites were used to quantify the in planta concentration of AR37-derived alkaloids through high-performance liquid chromatography.

2.3 | Determination of AR37 mycelial biomass within plants

The *Epichloë* biomass in plant shoots was determined using the amplification and quantification of the single copy fungal gene non-ribosomal peptide synthetase (NRPS1). Forward and reverse primers, GTCCGATCATTCCAAGCTCGTT and TGGTGGGAAGTTCCCTGCAC, amplified a PCR product of 153 bp (Rasmussen et al., 2007). The PCR product was quantified using a standard curve of the NRPS1 gene (10, 1, 0.1, 0.01 and 0.001 ng fungal DNA) that was prepared from DNA extracted from a pure culture of AR37. This DNA was extracted with the ZR Fungal/Bacterial DNA MiniPrep kit (Zymo Research Corp.). The qPCR was performed using LightCycler® 480 Sybr Green I Master (Roche Diagnostics) with 20 µL of reaction that contained total DNA (10–100 ng) and 200 nM of each primer. The *Epichloë* biomass was expressed as picograms of *Epichloë* DNA per nanogram of total (plant and *Epichloë*) DNA (i.e., pg ng^{-1} total DNA).

2.4 | Visualisation of AR37 hyphae within the elongation zone of leaf sheaths

The visualisation of *Epichloë* mycelia within vegetative leaf sheaths was performed with laser scanning confocal microscopy following the method described by Zhang et al. (2023). AR37 hyphal septa and cell walls were stained with wheat germ agglutinin-488 (light green) and aniline blue (cyan), and fluorescence signals were detected with a FluoView-FV10i confocal laser scanning microscope (Olympus Corp.).

2.5 | Characterisation of bacterial communities associated with the grass phyllosphere

Bacterial communities were characterised by sequencing the V4/V7 region within the bacterial 16S ribosomal RNA (rRNA) gene with an Illumina MiSeq instrument (paired end, 2 × 300 bp) (Illumina). Sequencing was performed on five randomly selected plant DNA samples from each endophyte and GA treatment combination at Days 3, 14 and 28 post-hormone application (60 samples in total). Libraries for sequencing were prepared by Macrogen Inc. using the primers 799F: AACMGGATTAGATACCCKG and 1193R: ACGTCATCCCCACCTTCC. These primers have been frequently used in metabarcoding studies and are highly specific for amplifying bacterial sequences in grasses (Liu, Tang, et al., 2022; Xia et al., 2023).

2.5.1 | Processing of sequencing data

Raw reads were processed following the DADA2 default pipeline (version 1.26) using the R software (version 4.2.2) (Callahan et al., 2016). Sequences were filtered and trimmed using the following setting: [truncLen = c (220, 220), maxEE = c (2, 2), truncQ = 2, maxN = 0, rm.phix = TRUE, multihread = TRUE]. Error learning and sequence inference were performed independently for each sequencing run as recommended, and sequence tables were merged using the mergeSequenceTables command. Chimeras were removed, and the DADA2 naïve Bayesian classifier method, coupled with the SILVA database v132.1, was used for the taxonomic identification of amplicon sequence variants (ASVs) (Wang et al., 2007).

Before downstream analyses, ASVs classified as chloroplast, mitochondria, nonbacterial taxa and with less than 10 reads were removed from the data set. Around 6% of the reads were discarded. The final data set included 1367 ASVs supported by ~2.72 million reads, with an average of 33 143 reads per sample (range 12 315−46 581 reads) (see Table S1). We opted not to rarefy the database to avoid discarding additional ASV/reads, and instead converted the read counts into relative abundance values. The rarefaction curves for all samples reached asymptotes (number of ASVs vs. number of reads), suggesting that the bacterial assemblies associated with the plant samples were well represented in our database (Supporting Information S1: Figure 1) (Wooley et al., 2010).

2.5.2 | Phylogenetic tree construction

The dominant bacterial ASVs in the phyllosphere were 149 (higher than 1% relative abundance, $n = 149$). The top 100 dominant bacterial ASVs were depicted in a dendrogram inferred with the MEGA X software (Kumar et al., 2018; Morella et al., 2020). The alignment of these 100 sequences was carried out with Multiple Alignments using the Fast Fourier Transform (MAFFT) programme running as a plug-in that is integrated within the Geneious software, with default parameters (Kearse et al., 2012). The phylogenetic reconstruction was performed using the Unweighted Pair Group Method with the Arithmetic mean (UPGMA) algorithm with a bootstrap test (9999 replicates) and default parameters (Hall, 2013).

2.6 | Quantification of AR37-derived alkaloids

Epoxyjanthitrem alkaloids (epoxyjanthitriol and epoxyjanthitrems I−IV) in extracted samples were quantified by comparison with the standard N-benzyl-1,8-naphthaleneimide (5 mg mL^{-1}), which had previously been compared with a pure epoxyjanthitrem I standard (Finch et al., 2020). Separation of a 5 µL injection was achieved using a Kinetex XB-C18 column (150 mm × 2.1 mm, 2.6 µm; Phenomenex Inc.) fitted with a SecurityGuardTM (2 mm × 3 mm C18 cartridge; Phenomenex Inc.) by gradient elution (0.4 mL min^{-1}) of 20 mM ammonium acetate in 10% acetonitrile (A) and acetonitrile (B) using the following gradient; 55% B at 0 min to 95% B at 13 min, held for 2 min, then returned to 55% B at 16 min and equilibrated for 4 min. Eluting compounds were detected by fluorescence (excitation wavelength: 333 nm; emission wavelength: 385 nm) with 0.1 µg g^{-1} of plant dry weight as the limit of quantification.

2.7 | Statistical analyses

The effect of the plant symbiotic status on the number of tillers and leaves before GA treatment were separately analysed with linear effect models, using the gls function from the nlme package in R software (assuming a normal distribution of errors) (Pinheiro et al., 2009). Models included the presence of *Epichloë* (E+, E−) as a categorical factor, and assumptions were met (residuals independence, normality and variance homogeneity). The effects of the plant symbiosis status and GA treatment on the plant height and aphid population sizes were separately assessed with linear mixed effect models, using the lme function from the same nlme package (assuming a normal distribution of errors). Models included the presence of *Epichloë* (E+, E−), GA treatment (GA+, GA−), time post-GA treatment (Days 3, 7, 14, 21 and 28) and the time nested in individual plants as a random effect. Temporal autocorrelations across repeated measurements were not observed. VarIdent variance structure was used in the endophyte × time and GA × time interactions to minimise dependency in residuals in plant height and aphid population size variables (Zuur et al., 2009). After that, the model assumptions were met (residuals independence, normality and variance homogeneity).

The effects of the GA treatment on the alkaloid concentration and fungal biomass were separately analysed with linear mixed effect models, also using the lme function from the nlme package (assuming a normal distribution of errors). Models included the GA treatment

(GA+, GA−), time post-GA treatment (Days 3, 7, 14, 21 and 28) and the time nested in individual plants as a random effect. Temporal autocorrelations across repeated measurements were not observed. VarIdent variance structure was used in the time factor to minimise dependency in residuals in alkaloid concentration and fungal biomass variables. After that, the model assumptions were met.

The effect of the plant symbiosis status and GA treatment on the composition of bacterial assemblages of plant tillers were analysed using permutational multivariate analysis of variance (PERMANOVA) (999 permutations), based on Bray−Curtis distances, with the function adonis2 from the vegan R package (Anderson, 2001; Oksanen et al., 2020). PERMANOVA allows testing for differences between groups when multivariate responses are measured. The model included the presence of *Epichloë* (E+, E−), GA treatment (GA+, GA−) and the time post-GA treatment (Days 3, 14, 28) as categorical factors. The strata term was used to limit the permutations within each individual plant to account for the repeated measures (Oksanen et al., 2020). To characterise the composition differences between bacterial assemblages associated with plant tillers, nonmetric multidimensional scaling (NMDS) based on Bray−Curtis dissimilarities were performed using the vegan R package. A Shepard diagram correlating NMDS ordination distances and Bray−Curtis community dissimilarities was used to validate NMDS ordination analysis. A high correlation indicates that the calculated NMDS ordination is a good representation of community structures (Leeuw & Mair, 2015). The effects of the treatments on the relative abundance of dominant phyllosphere bacterial taxa ($n = 149$) were analysed with the *DESeq* function from the DESeq2 package in the R software (assuming the negative binomial distribution of errors) (Love et al., 2014). All the presented values in the result section are means ± standard errors of the mean. All the analyses were performed on the R software, version 4.2.2 (R Core Team, 2022).

3 | RESULTS

3.1 | Effect of AR37 on plant growth prior to the GA treatment

Prior to the GA treatment, the AR37 presence affected the growth of their plant hosts (Table 1), increasing the number of tillers and leaves by 33% each (Figure 1a,b).

3.2 | Effects of AR37 and GA treatment on plant growth and resistance to insects

As expected, plant height was affected by the GA treatment, and this effect varied with the experimental time (GA × time) and was independent on the presence of AR37 (Table 1). The GA treatment increased the plant height from Day 7 post-hormone application, and this increment was as high as 18% at Day 28 post-hormone application (Figure 2a). The effect of the AR37 presence on aphid population sizes depended on the plant GA treatment and varied with the experimental time (endophyte × GA × time) (Table 1). In agreement with our hypothesis, from Day 21 post-hormone treatment, E+ plants showed reduced aphid population sizes irrespective of GA application, while E− plants harboured increased aphid population sizes, with the highest population sizes recorded in E−/GA+ plants (Figure 2b). At Day 28, the sizes of aphid populations were 60% lower in E+ plants than in E− plants, and GA treatment increased the number of aphids in the E− plants by 61% (Figure 2b).

3.3 | Effect of GA treatment on AR37-derived alkaloid concentrations, mycelial biomass of AR37 and hyphal integrity of AR37

The concentration of AR37-derived epoxyjanthitrems in plants was affected by the interaction between GA treatment and experimental time (Table 1). While the concentration of these alkaloids did not vary in most of the insect trials, at Day 28 post-GA treatment, E+ plants showed increased epoxyjanthitrem concentrations, and the increase was more pronounced in the GA− than GA+ symbiotic plants (Figure 3).

The AR37 mycelial biomass in plants was also affected by the interaction between GA treatment and experimental time (Table 1). While *Epichloë* mycelial biomass did not vary in most of the insect trials, at Day 14 post-GA treatment, E+ plants treated with GA exhibited twice as much *Epichloë* mycelial biomass than GA-untreated plants (Figure 4a). Microscopic examination of leaf sheaths at Day 14 showed that the integrity of the *Epichloë* hyphae was affected by the GA treatment. In GA-treated plants, fungal hyphae of AR37 were broken at and between the septa, relatively thinner in diameter, or with reduced cell walls (Figure 4b). Fungal hyphae of AR37 were intact in GA-untreated plants (Figure 4b and Supporting Information S1: Figure 2).

3.4 | Effects of AR37 and GA treatment on the composition of phyllosphere bacterial communities and relative abundance of individual taxa

The composition of the bacterial communities in the grass phyllosphere was influenced by the presence of AR37 and varied across experimental time (i.e., endophyte × time), but contrary to our prediction, the composition was independent of the GA treatment and associated interactions (e.g., GA × time) (Table 1). These effects were visualised in NMDS ordinations that showed that bacterial communities were grouped by the plant endophyte status rather than GA treatment and that the spatial distribution of bacterial communities associated with E+ and E− plants changed over time. The temporal variation was more pronounced between Days 14 and 28 than 3 and 14 post-GA treatment in which bacterial communities in E+ and E− plants occupied opposite spatial positions along the NMDS 1 axis (Figure 5). We conducted further analyses on the relative

TABLE 1 Effects of *Epichloë* sp. LpTG-3 strain AR37 status, gibberellin (GA) treatment and experimental time on the distinct response variables that were measured in ryegrass plants.

Response variable	Source of variation	*df*1	*df*2	*F*-value	*p* Value
Tiller number prior to GA application (#·plant^{-1})	Endophyte	1	38	7.92	**0.007**
Leaf number prior to GA application (#·plant^{-1})	Endophyte	1	38	7.75	**0.008**
Plant height (cm)	Endophyte	1	36	0.00	0.982
	GA	1	36	63.17	**<0.001**
	Time	4	144	830.22	**<0.001**
	Endophyte × GA	1	36	3.99	0.053
	Endophyte × time	4	144	0.11	0.978
	GA × time	1	36	11.51	**<0.001**
	Endophyte × GA × time	4	144	0.58	0.672
Aphid population size (#·plant^{-1})	Endophyte	1	36	27.39	**<0.001**
	GA	1	36	0.35	0.557
	Time	3	108	263.99	**<0.001**
	Endophyte × GA	1	36	3.48	0.070
	Endophyte × time	3	108	54.15	**<0.001**
	GA × time	1	36	7.97	**<0.001**
	Endophyte × GA × time	3	108	10.17	**<0.001**
Epoxyjanthitrems (μg g^{-1} DW)	GA	1	18	0.95	0.34
	Time	4	72	25.87	**<0.001**
	GA × time	4	72	2.93	**0.026**
Epichloë mycelial biomass (pg ng^{-1} DNA)	GA	1	18	1.52	0.233
	Time	4	72	16.54	**<0.001**
	GA × time	4	72	2.56	**0.045**
Bacterial composition	Endophyte	1	52	2.14	**0.013**
	GA	1	52	1.23	0.210
	Time	1	52	2.51	**0.008**
	Endophyte × GA	1	52	1.58	0.059
	Endophyte × time	1	52	3.75	**0.001**
	GA × time	1	52	1.02	0.381
	Endophyte × GA × time	1	52	0.89	0.558

Note: Statistically significant effects are highlighted in bold in the '*p*-value' column.
Abbreviation: DW, dry weight.

abundance of bacterial taxa at Days 14 and 28 post-GA treatment. E+ plants were dominated by a few bacterial taxa compared to E− at Day 14 post-GA treatment, while the opposite occurred at Day 28 post-GA treatment (Figure 6). The bacterial taxa that explained most of the changes in bacterial communities between Days 14 and 28 post-GA treatment were *Pseudomonas*, *Xanthomonas*, *Luteibacter*, *Curtobacterium* and *Agreia* (Figure 6) (see Table S2 for the statistics associated with the taxa comparisons).

4 | DISCUSSION

This study evaluated the effects of *Epichloë* presence and GA treatment on plant growth, plant resistance to aphid herbivores, *Epichloë*-derived alkaloid concentrations, *Epichloë* mycelial biomass, hyphal integrity and phyllosphere bacterial communities in a time course experiment. As documented in other plant-*Epichloë* symbioses (Bastías et al., 2021; Gundel et al., 2013), the presence of AR37

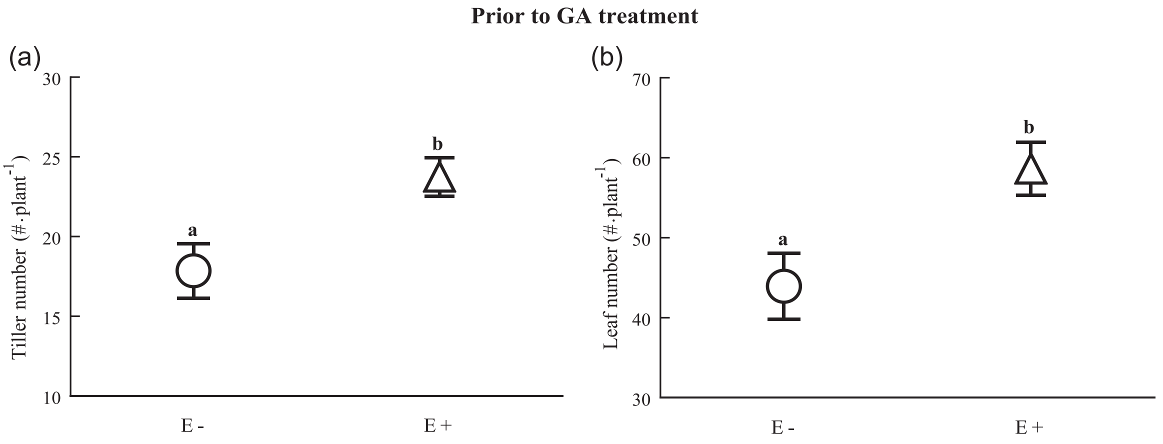

FIGURE 1 Number of tillers (a) and leaves (b) in 5-month-old ryegrass plants with (E+, triangles) and without (E−, circles) *Epichloë* sp. LpTG-3 strain AR37 prior to the gibberellin (GA) treatment. Letters indicate statistically significant differences. Values are mean ± SEM values ($n = 20$). SEM, standard errors of the mean.

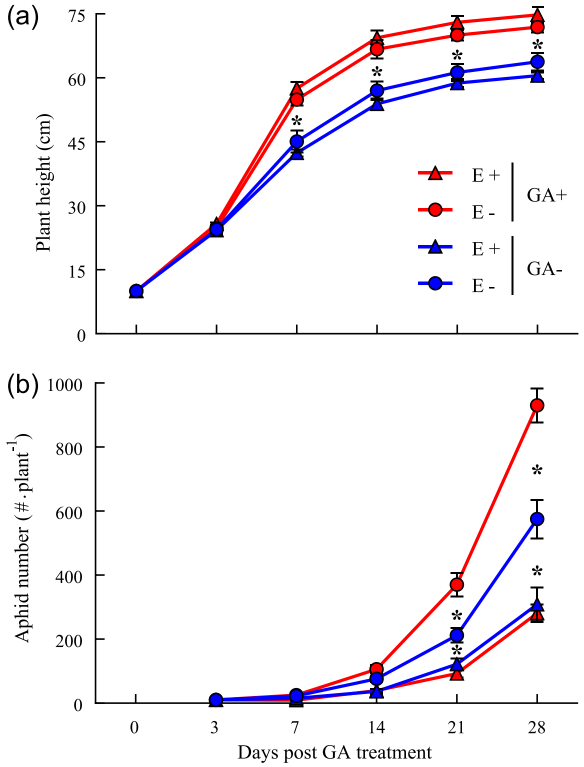

FIGURE 2 Height (a) and population sizes of *Rhopalosiphum padi* aphids (b) in 5-month-old ryegrass plants with (E+, triangles) and without (E−, circles) *Epichloë* sp. LpTG-3 strain AR37 over time post the gibberellin treatment (treated: GA+, red lines; untreated: GA−, blue lines). Asterisks indicate statistically significant differences in relevant comparisons for the study. Ten *R. padi* aphids were added to each plant 3 days post-GA application. Values are mean ± SEM values ($n = 10$). GA, gibberellin; SEM, standard errors of the mean.

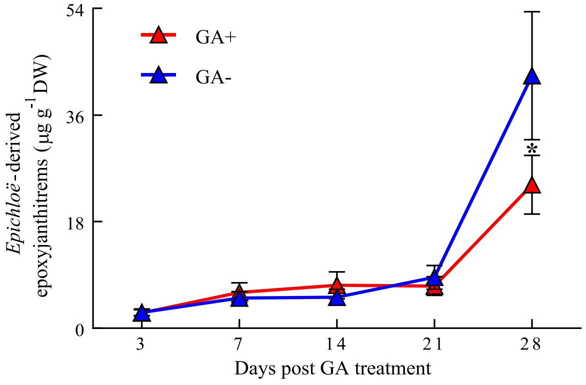

FIGURE 3 The concentration of epoxyjanthitrems (epoxyjanthitrems I–IV + epoxyjanthitriol) in 5-month-old ryegrass plants associated with *Epichloë* sp. LpTG-3 strain AR37 over time post the gibberellin treatment (treated: GA+, red line; untreated: GA−, blue line). Asterisks indicate statistically significant differences in relevant comparisons for the study. Ten *Rhopalosiphum padi* aphids were added to each plant 3 days post-GA application. Values are mean ± SEM values ($n = 10$). GA, gibberellin; SEM, standard errors of the mean.

increased the size (tiller and leaf numbers) of their plant hosts. We predicted that the GA-associated growth promotion would reduce the resistance to aphids in E− plants but would not compromise the resistance in E+ plants due to the production of *Epichloë*-derived alkaloids. In agreement with the prediction, the treatment with GA promoted the growth of plants by increasing plant height. Most importantly, the hormone increased the susceptibility to aphids in E− plants but did not alter the aphid resistance in E+ plants. As expected, the GA treatment did not affect the concentration of AR37-derived alkaloids during most of the insect trials and when affected (i.e., Day 28 post-GA application), these compounds were increased above the observed average concentrations. Furthermore, the GA treatment did not alter the mycelial biomass of AR37 during most of the insect trials, except at Day 14 post-GA treatment, where fungal biomass increased, but at the expense of hyphal integrity. We also predicted that both GA treatment, via crosstalk suppression of host defences, and *Epichloë* presence in plants would affect the phyllosphere bacterial microbiota. Only the presence of *Epichloë* in

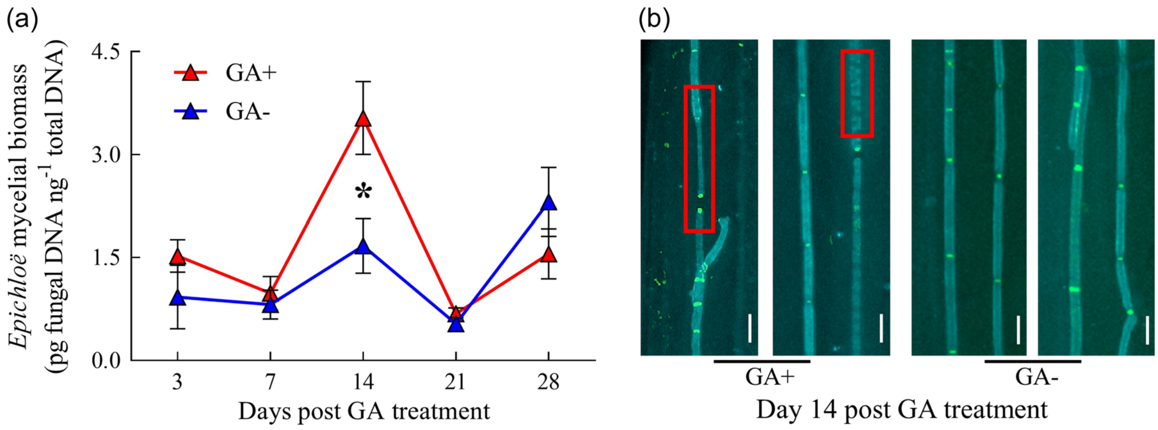

FIGURE 4 *Epichloë* mycelial biomass (a) and hyphal integrity (b) in 5-month-old ryegrass plants associated with *Epichloë* sp. LpTG-3 strain AR37 over time or at Day 14 post the gibberellin treatment (treated: GA+, red line; untreated: GA−, blue line). Asterisks indicate statistically significant differences in relevant comparisons for the study. Ten *Rhopalosiphum padi* aphids were added to each plant 3 days post-GA application. Values are mean ± SEM values ($n = 10$) for (a). Hyphal septa were stained with wheat germ agglutinin-488 (light green), while fungal cell walls were stained with aniline blue (cyan). Red rectangles show broken, thin or degraded hyphae. The scale bar is equal to 10 µm. GA, gibberellin; SEM, standard errors of the mean. [Color figure can be viewed at wileyonlinelibrary.com]

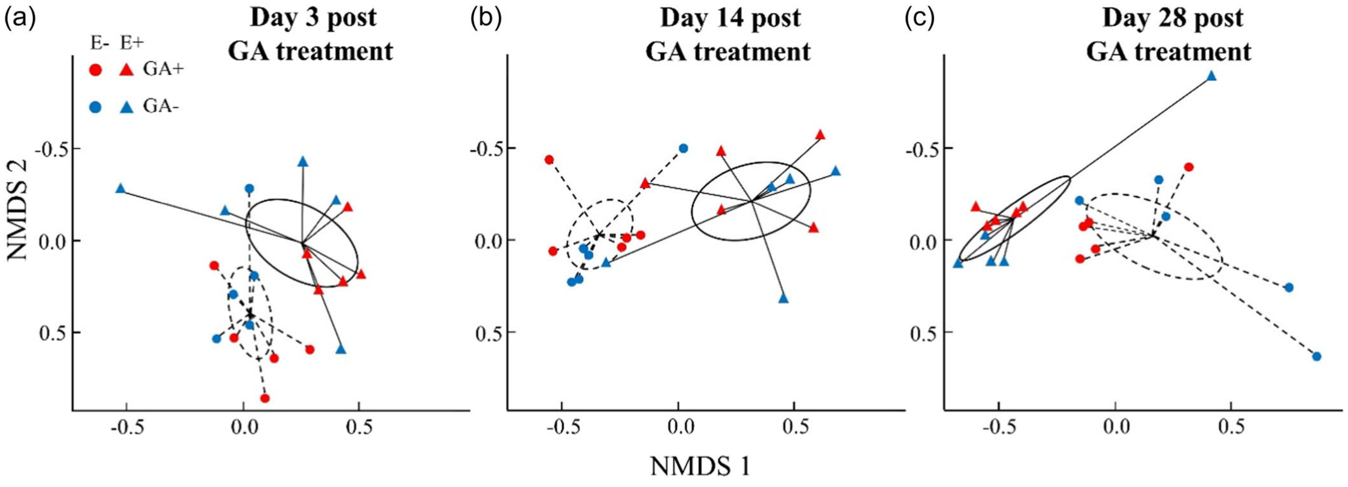

FIGURE 5 Nonmetric multidimensional scaling ordinations (NMDS; stress = 0.15) for bacterial assemblages associated with the phyllosphere of 5-month-old ryegrass plants with (E+, triangles) and without (E−, circles) *Epichloë* sp. LpTG-3 strain AR37 at Days 3 (a), 14 (b), and 28 (c) post the gibberellin treatment (treated: GA+, red symbols; untreated: GA−, blue symbols). Lines indicate bacterial compositional distances between centroids and E+ and E− plants (continuous and discontinuous lines, respectively). Ellipses represent 95% confidence intervals around centroids and show clustering of bacterial compositions in plants based on the presence/absence of AR37 (continuous and discontinuous lines, respectively). Ten *Rhopalosiphum padi* aphids were added to each plant 3 days post-GA application. The Shepard diagram (Supporting Information S1: Figure 3) showed a good linear fit between ordination distances and Bray−Curtis dissimilarities ($n = 5$). GA, gibberellin; SEM, standard errors of the mean. [Color figure can be viewed at wileyonlinelibrary.com]

plants, and not the GA treatment, altered the composition of the bacterial microbiota, with stronger and contrasting effects between Days 14 and 28 post-GA treatment. The presence of *Epichloë* mainly affected *Pseudomonas*, *Xanthomonas*, *Luteibacter*, *Curtobacterium* and *Agreia* taxa.

The growth-defence trade-off is ubiquitous in plants, including grasses (Herms & Mattson, 1992; Lind et al., 2013). Our results showed that this phenomenon was indeed present in the E− plants, that is, the GA-based growth stimulation of E− plants made plants more susceptible to aphids. We predicted that grasses associated with *Epichloë* endophytes would not display the trade-off. Our results agreed with this prediction, that is, the GA-based growth promotion in E+ plants did not alter the degree of resistance of plants to aphids. We predicted that the absence of the trade-off in E+ plants would be associated with the fungal provision of alkaloids that circumvent the requirement for the plant's own defences. The AR37-derived epoxyjanthitrems generally exhibit strong bioactivity against insect herbivores (Finch et al., 2020). Our results confirmed that the GA treatment did not affect the concentration of these alkaloids during most of the insect trials. Therefore, it is reasonable to posit that the

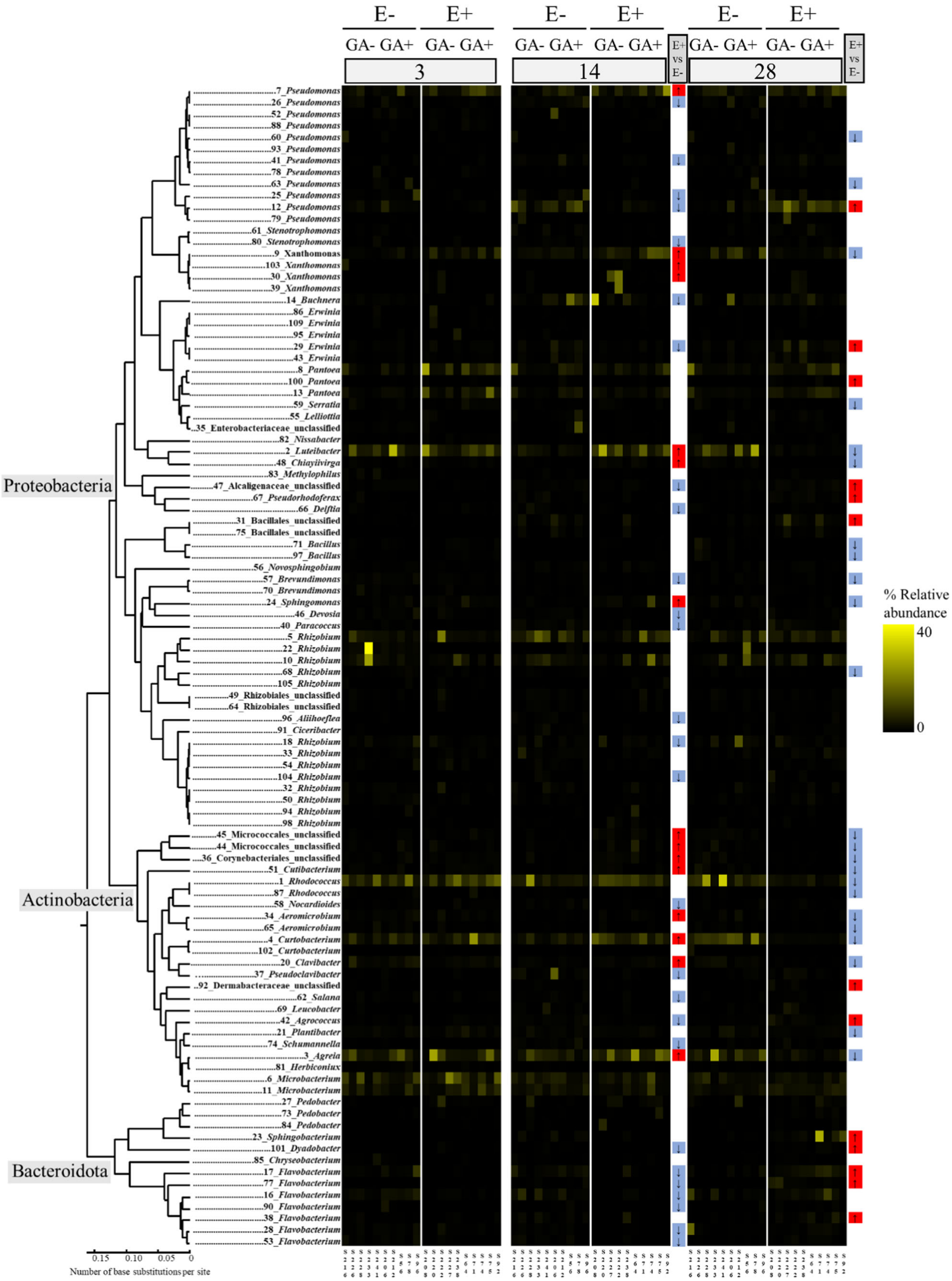

FIGURE 6 (See caption on next page).

expected reduction of the plant's own defence response, due to the hormonal treatment, was compensated by the action of antiherbivore alkaloids, whose concentrations were mostly unaffected by the GA treatment. Collectively, these findings clearly demonstrate that *Epichloë* does indeed relieve the trade-off between growth and defence responses of their plant hosts and that the decoupling mechanism is likely to be associated with the presence of *Epichloë*-derived antiherbivore alkaloids in plants. Future experiments with endophytes lacking the production of these secondary metabolites are needed to confirm the mechanism (see also Bastías et al., 2021).

The growth of *Epichloë* within expanding leaf sheaths of grasses occurs when the expansion of plant cells exerts mechanical stretch on the attached fungal hyphae leading to hyphal extension, which then promotes the compartmentalisation of fungal hyphae via nuclear division and septation (Ariyawansa, 2015; Scott et al., 2018; Voisey, 2010). The rapid elongation of plant cells is likely to exert high mechanical stretch on *Epichloë* hyphae, and this might occur, for instance, in situations where grasses are competing for light, where shoot elongation is generally stimulated (Casal et al., 1987). In the present experiment, GA-mediated enhancement of the elongation rate of plant cells might have occurred since the exhibited gain in plant height was higher in GA-treated compared to GA-untreated plants, especially at Days 3 and 14 post-hormone application (i.e., the slope of plant height day^{-1} was steeper in GA+ than GA− plants between these days). An increased elongation rate in plant cells is expected after exogenous applications of GA (Sauret-Güeto et al., 2012). This increased elongation rate likely increased the mechanical stretch on fungal hyphae leading to hyphal sections with fragmentations and large reductions in diameter. The high mycelial biomass observed in GA+ plants at Day 14 should have resulted from the enhanced cell proliferation that is expected from stretched hyphae (Scott et al., 2018). However, we speculate that *Epichloë* hyphal growth could not match the fast growth of the plant host when treated with GA, and this led to a breakage in the observed hyphae (Noorifar et al., 2021). The degradation of *Epichloë* hyphal cells observed at Day 14 post-GA may be linked to the reduction in mycelial biomass at Day 21 post-GA treatment. The notion that increases in *Epichloë* mycelial biomass is driven by the mechanical stretch exerted by the plant on the fungus is further supported by the absence of further increases in mycelial biomass at the end of the insect trial when plants were growing slower (i.e., the GA+ and GA− plant height day^{-1} slopes were similar and flat from Day 21 post-hormone application). Importantly for the endophyte-mediated trade-off decoupling hypothesis, our results suggest that reductions in hyphal integrity do not necessarily imply a loss in the *Epichloë* ability to decouple the plant growth-defence trade-off (i.e., E+ plants were still resistant to aphids despite the loss in hyphal integrity at Day 14 post-GA treatment). The evidence regarding broken hyphae within plant tissues in the present study reaffirms the relevance of the growth synchronisation between the fungus and the plant (Scott et al., 2018).

A positive relationship exists between *Epichloë* mycelial biomass and the concentration of some *Epichloë*-derived alkaloids (e.g., Freitas et al., 2020; Rasmussen et al., 2007). This positive relationship was observed in our system, especially towards the end of the insect trial. From Day 21 to 28 post-GA treatment, the overall increase in *Epichloë* mycelial biomass was associated with enhanced levels of epoxyjanthitrems. The high levels of mycelial biomass and fungal-derived alkaloids at the end of the insect trial might be associated with the fact that plants were entering into the reproductive stage (Ball et al., 1997; May et al., 2008). It is interesting to note that at Day 14 post-GA treatment, the enhanced *Epichloë* mycelial biomass did not result in increased concentrations of epoxyjanthitrems in plant tissues, possibly due to damaged hyphae as described above. Less of an increase in the concentration of epoxyjanthitrems was observed in GA+ compared to GA− plants at Day 28 post-hormal application. Since no changes in fungal biomass were observed at this timepoint, a potential explanation could be related to a potential 'dilution' effect (Rasmussen et al., 2007). GA treatment could induce high increases in plant cell diameter and plant cell wall thickness of fibres in the host plants over time (Stant, 1963). At Day 28 post-GA treatment, when plants were entering the reproductive stage with significant increases in alkaloid production, the dry mass of GA+ plants were likely greater than that of GA− plants, thus diluting the concentration of *Epichloë*-derived alkaloids in GA+ plants.

We expected that the hormone treatment would influence the bacterial microbiota since the GA-associated suppression of plant JA defence responses would make plants susceptible to certain microbial groups such as pathogens (Pfeilmeier et al., 2021). Our result showed that the bacterial microbiota of the plant phyllosphere was not enriched with any particular bacterial group due to the GA treatment. Additionally, plant shoots did not show signals

FIGURE 6 Phylogenetic tree, relative abundance and differentially expressed taxa of the top 100 dominant bacterial ASVs (amplicon sequence variants) in the phyllosphere of 5-month-old ryegrass plants with (E+) and without (E−) *Epichloë* sp. LpTG-3 strain AR37 at Days 3, 14 and 28 post-gibberellin treatment (treated: GA+; untreated: GA). The tree was inferred using the Unweighted Pair Group Method with Arithmetic mean and evolutionary distances calculated using the Maximum Composite Likelihood method. The heat map represents the relative abundances of dominant bacterial ASV (>1% relative abundance). Dominant bacterial ASVs represented 69%–97% of the abundances of sequence reads associated with each sample. Bacterial taxa with significantly different relative abundances in E+ versus E− plants are indicated in red and blue cells (see the statistics associated with taxa comparisons in Table S2). This analysis was restricted to Days 14 and 28 post-GA treatment since bacterial composition showed major differences due to the presence of *Epichloë* sp. AR37 at these time points. The '↑' symbol in red cells indicates that a bacterial taxon abundance was positively associated with the presence of AR37, while the '↓' symbol in blue cells means the opposite was observed. Sample identifications are indicated at the bottom of the heat map. Ten *Rhopalosiphum padi* aphids were added to each plant 3 days post-GA application. GA, gibberellin. [Color figure can be viewed at wileyonlinelibrary.com] of pathogen infection across the whole experiment. It is challenging to explain this result considering that the GA treatment effectively reduced the plant's own defences as suggested by the aphid population results in the E− plants. It is possible that other plant defence mechanisms, certainly not JA, with effectiveness just against bacterial microbes but not against aphids, contributed to maintaining a largely unaltered phyllosphere bacterial microbiota in GA+ and GA− plants (Felton et al., 1999). For instance, it has been documented that the GA pathway can stimulate the expression of plant SA defence responses, and this stimulation increased the plant resistance level against pathogens (Navarro et al., 2008; De Vleesschauwer et al., 2016). Alternatively, it is not possible to discard the possibility that the root microbiota could have been affected by the hormonal treatment (Tang et al., 2022). *Epichloë* AR37 influenced the phyllosphere bacterial microbiota, and this agrees with previously documented experimental results (e.g., Bastías, Bustos, et al., 2022; Liu, Tang, et al., 2022). This is also in agreement with another experimental findings that showed that *Epichloë* altered root-associated bacterial communities and shoot- or root-associated fungal communities (Jin, Wang, et al., 2023; Nissinen et al., 2019; Rasmussen et al., 2023; Zhong et al., 2018) (but see e.g., Dale & Newman, 2022). Our results showed that the most substantial *Epichloë*-based changes in bacterial composition occurred at Days 14 and 28 post-GA treatment. At Day 14, E+ were dominated by a few bacterial taxa compared to E− plants, but this pattern was reversed at Day 28, where bacterial taxa were more evenly distributed in E+ than in E− plants. Increased alkaloid concentrations at the end of the insect trial could explain the changes in the phyllosphere bacterial microbiota. The presence of alkaloids in foliar tissues has been documented to affect the phyllosphere bacterial microbiota since certain bacterial taxa use alkaloids as a nutrient source (Roberts & Lindow, 2014). For instance, members within the *Pseudomonas* group can metabolise certain *Epichloë*-derived alkaloids, and in fact, in our experiment, the E+ phyllosphere was enriched with a member of this group at the end of the insect trial (Roberts & Lindow, 2014). It is therefore reasonable to posit that a potential exclusion of bacterial groups that were unable to metabolise the enhanced alkaloid contents at the end of the insect trial had contributed to increasing the evenness in the E+ plants.

5 | CONCLUSION AND FUTURE PERSPECTIVES

A debate has been raised regarding whether fungal endophytes can decouple the growth-defence trade-off of plants (e.g., Bastías, Gundel, et al., 2022). Results of the present study demonstrate that the presence of *Epichloë* endophytes in plants alleviated the growth-defence trade-off of their hosts: the GA-mediated penalisation of plant defences was observed in the endophyte-free but not in the endophyte-symbiotic plants. These experimental results are in agreement with outcomes from a meta-analysis of published literature that included several plant-endophyte symbioses (Bastías et al., 2021). We have posited that the presence of antiherbivore alkaloids would be the trade-off decoupling mechanism in plant-*Epichloë* associations. The recognised bioactivity of AR37-derived alkaloids against insects, along with the lack of negative effects of the GA treatment on the alkaloid levels, strongly suggest that these compounds were the mechanism driving the endophyte-mediated trade-off decoupling. However, the unequivocal confirmation of the mechanism might come from experiments that manipulate the alkaloid levels. The use of plants associated with gene-edited endophytes with decreased and no production of alkaloids would be an elegant system to evaluate the endophyte-mediated mechanism of the trade-off decoupling and also to quantify the costs associated with this mechanism (Hudson et al., 2021; Miller et al., 2022). Further experiments could also investigate the changes in the phytohormonal networks and plant resource levels associated with the ability of endophytes to alleviate the trade-off. These investigations might decipher if the endophyte-mediated ability to decouple the trade-off is based on a phytohormonal regulation or reallocation of plant resources (Monson et al., 2022).

ACKNOWLEDGEMENTS

We would like to thank David Hume and Zac Beechey-Gradwell for the critical revision of earlier versions of the manuscript. We thank Yulia Morozova (AgResearch Ltd.) for performing alkaloid measurements. This work was supported by the Strategic Science Investment Fund (A20067) and Endeavour Fund (LVLX1702) from the New Zealand Ministry of Business, Innovation and Employment (MBIE), and by the Fondo Nacional de Desarrollo Científico of Chile granted to PEG (FONDECYT-2021-1210908). Open access publishing facilitated by AgResearch Ltd, as part of the Wiley - AgResearch Ltd agreement via the Council of Australian University Librarians.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article. The microbiota data presented in the study are deposited in the NCBI Short Read Archive (SRA), accession number PRJNA1065566.

ORCID

Wei Zhang http://orcid.org/0000-0003-2959-4482
Pedro E. Gundel http://orcid.org/0000-0003-3246-0282
Ruy Jáuregui http://orcid.org/0000-0002-3484-8937
Stuart D. Card http://orcid.org/0000-0001-7140-7471
Wade J. Mace https://orcid.org/0000-0002-3529-7700
Richard D. Johnson https://orcid.org/0000-0003-2100-6658
Daniel A. Bastías http://orcid.org/0000-0002-0522-5538

REFERENCES

Altmann, M., Altmann, S., Rodriguez, P.A., Weller, B., Elorduy Vergara, L., Palme, J. et al. (2020) Extensive signal integration by the phytohormone protein network. *Nature*, 583, 271–276.

Anderson, M.J. (2001) A new method for non-parametric multivariate analysis of variance. *Austral Ecology*, 26, 32–46.

Ariyawansa, K. (2015) Sensing and signalling mechanical stress during intercalary growth in *Epichloë* grass endophytes. Massey University. http://hdl.handle.net/10179/7750

Ballaré, C.L. & Austin, A.T. (2019) Recalculating growth and defense strategies under competition: key roles of photoreceptors and jasmonates. *Journal of Experimental Botany*, 70, 3425–3434.

Ball, O.J.-P., Barker, G.M., Prestidge, R.A. & Sprosen, J.M. (1997) Distribution and accumulation of the mycotoxin lolitrem B in *Neotyphodium lolii*-infected perennial ryegrass. *Journal of Chemical Ecology*, 23, 1435–1449.

Bastías, D.A., Balestrini, R., Pollmann, S. & Gundel, P.E. (2022) Environmental interference of plant-microbe interactions. *Plant, Cell & Environment*, 45, 3387–3398.

Bastías, D.A., Bustos, L.B., Jáuregui, R., Barrera, A., Acuña-Rodríguez, I.S., Molina-Montenegro, M.A. et al. (2022) *Epichloë* fungal endophytes influence seed-associated bacterial communities. *Frontiers in Microbiology*, 12, 795354.

Bastías, D.A., Gianoli, E. & Gundel, P.E. (2021) Fungal endophytes can eliminate the plant growth–defence trade-off. *New Phytologist*, 230, 2105–2113.

Bastías, D.A. & Gundel, P.E. (2023) Plant stress responses compromise mutualisms with *Epichloë* endophytes. *Journal of Experimental Botany*, 74, 19–23.

Bastías, D.A., Gundel, P.E., Johnson, R.D. & Gianoli, E. (2022) How and when fungal endophytes can eliminate the plant growth–defence trade-off: mechanistic perspectives. *New Phytologist*, 235, 388–390.

Bastias, D.A., Martínez-Ghersa, M.A., Ballaré, C.L. & Gundel, P.E. (2017) *Epichloë* fungal endophytes and plant defenses: not just alkaloids. *Trends in Plant Science*, 22, 939–948.

Bastías, D.A., Martínez-Ghersa, M.A., Newman, J.A., Card, S.D., Mace, W.J. & Gundel, P.E. (2018) The plant hormone salicylic acid interacts with the mechanism of anti-herbivory conferred by fungal endophytes in grasses. *Plant, Cell & Environment*, 41, 395–405.

Bastías, D.A., Martínez-Ghersa, M.A., Newman, J.A., Card, S.D., Mace, W.J. & Gundel, P.E. (2019) *Sipha maydis* sensitivity to defences of *Lolium multiflorum* and its endophytic fungus *Epichloë occultans*. *PeerJ*, 7, e8257.

De Battista, J.P., Bacon, C.W., Severson, R., Plattner, R.D. & Bouton, J.H. (1990) Indole acetic acid production by the fungal endophyte of tall fescue. *Agronomy Journal*, 82, 878–880.

Bennett, A.E., Alers-Garcia, J. & Bever, J.D. (2006) Three-way interactions among mutualistic mycorrhizal fungi, plants, and plant enemies: hypotheses and synthesis. *The American Naturalist*, 167, 141–152.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A. & Holmes, S.P. (2016) DADA2: high-resolution sample inference from Illumina amplicon data. *Nature Methods*, 13, 581–583.

Campos, M.L., Yoshida, Y., Major, I.T., de Oliveira Ferreira, D., Weraduwage, S.M., Froehlich, J.E. et al. (2016) Rewiring of jasmonate and phytochrome B signalling uncouples plant growth-defense tradeoffs. *Nature Communications*, 7, 12570.

Caradus, J.R. & Johnson, L.J. (2020) *Epichloë* fungal endophytes-From a biological curiosity in wild grasses to an essential component of resilient high performing ryegrass and fescue pastures. *Journal of Fungi*, 6, 322.

Casal, J.J., Sánchez, R.A. & Deregibus, V.A. (1987) The effect of light quality on shoot extension growth in three species of grasses. *Annals of Botany*, 59, 1–7.

Christensen, M.J., Bennett, R.J., Ansari, H.A., Koga, H., Johnson, R.D., Bryan, G.T. et al. (2008) *Epichloë* endophytes grow by intercalary hyphal extension in elongating grass leaves. *Fungal Genetics and Biology*, 45, 84–93.

Dale, J.C.M. & Newman, J.A. (2022) A first draft of the core fungal microbiome of *Schedonorus arundinaceus* with and without its fungal mutualist *Epichloë coenophiala*. *Journal of Fungi*, 8, 1026.

Denancé, N., Sánchez-Vallet, A., Goffner, D. & Molina, A. (2013) Disease resistance or growth: the role of plant hormones in balancing immune responses and fitness costs. *Frontiers in Plant Science*, 4, 155.

Dupont, P.Y., Eaton, C.J., Wargent, J.J., Fechtner, S., Solomon, P., Schmid, J. et al. (2015) Fungal endophyte infection of ryegrass reprograms host metabolism and alters development. *New Phytologist*, 208, 1227–1240.

Eichmann, R., Richards, L. & Schäfer, P. (2021) Hormones as go-betweens in plant microbiome assembly. *The Plant Journal*, 105, 518–541.

Felton, G.W., Korth, K.L., Bi, J.L., Wesley, S.V., Huhman, D.V., Mathews, M.C. et al. (1999) Inverse relationship between systemic resistance of plants to microorganisms and to insect herbivory. *Current Biology*, 9, 317–320.

Finch, S.C., Prinsep, M.R., Popay, A.J., Wilkins, A.L., Webb, N.G., Bhattarai, S. et al. (2020) Identification and structure elucidation of epoxyjanthitrems from *Lolium perenne* infected with the endophytic fungus *Epichloë festucae* var. *lolii* and determination of the tremorgenic and anti-insect activity of epoxyjanthitrem I. *Toxins*, 12, 526.

Freitas, P.P., Hampton, J.G., Rolston, M.P., Glare, T.R., Miller, P.P. & Card, S.D. (2020) A tale of two grass species: temperature affects the symbiosis of a mutualistic *Epichloë* endophyte in both tall fescue and perennial ryegrass. *Frontiers in Plant Science*, 11, 530.

Graff, P., Gundel, P.E., Salvat, A., Cristos, D. & Chaneton, E.J. (2020) Protection offered by leaf fungal endophytes to an invasive species against native herbivores depends on soil nutrients. *Journal of Ecology*, 108, 1592–1604.

Gundel, P.E., Pérez, L.I., Helander, M. & Saikkonen, K. (2013) Symbiotically modified organisms: nontoxic fungal endophytes in grasses. *Trends in Plant Science*, 18, 420–427.

Gundel, P.E., Rudgers, J.A. & Ghersa, C.M. (2011) Incorporating the process of vertical transmission into understanding of host–symbiont dynamics. *Oikos*, 120, 1121–1128.

Guo, H., Nolan, T.M., Song, G., Liu, S., Xie, Z., Chen, J. et al. (2018) FERONIA receptor kinase contributes to plant immunity by suppressing jasmonic acid signaling in *Arabidopsis thaliana*. *Current Biology*, 28, 3316–3324.e6.

Hahn, H., McManus, M.T., Warnstorff, K., Monahan, B.J., Young, C.A., Davies, E. et al. (2008) Neotyphodium fungal endophytes confer physiological protection to perennial ryegrass (*Lolium perenne* L.) subjected to a water deficit. *Environmental and Experimental Botany*, 63, 183–199.

Hall, B.G. (2013) Building phylogenetic trees from molecular data with MEGA. *Molecular Biology and Evolution*, 30, 1229–1235.

Hennessy, L.M., Popay, A.J., Finch, S.C., Clearwater, M.J. & Cave, V.M. (2016) Temperature and plant genotype alter alkaloid concentrations in ryegrass infected with an *Epichloë* endophyte and this affects an insect herbivore. *Frontiers in Plant Science*, 7, 1097.

Herms, D.A. & Mattson, W.J. (1992) The dilemma of plants: to grow or defend. *The Quarterly Review of Biology*, 67, 283–335.

He, Z., Webster, S. & He, S.Y. (2022) Growth–defense trade-offs in plants. *Current Biology*, 32, R634–R639.

Horváth, E., Pál, M., Szalai, G., Páldi, E. & Janda, T. (2007) Exogenous 4-hydroxybenzoic acid and salicylic acid modulate the effect of short-term drought and freezing stress on wheat plants. *Biologia Plantarum*, 51, 480–487.

Hudson, D., Mace, W., Popay, A., Jensen, J., McKenzie, C., Cameron, C. et al. (2021) Genetic manipulation of the ergot alkaloid pathway in *Epichloë festucae* var. *lolii* and its effect on black beetle feeding deterrence. *Toxins*, 13, 76.

Jin, G., Qi, J., Zu, H., Liu, S., Gershenzon, J., Lou, Y. et al. (2023) Jasmonate-mediated gibberellin catabolism constrains growth during herbivore attack in rice. *The Plant Cell*, 35, 3828–3844.

Jin, J., Wang, J., Niu, X., Wang, C., Malik, K. & Li, C. (2023) Integrated microbiology and metabolomics analysis reveal patterns and mechanisms of improvement the *Achnatherum inebrians* adaptability to N addition by endophytic fungus *Epichloë gansuensis*. *Environmental and Experimental Botany*, 213, 105421.

Johnson, L.J., Bastías, D.A., Caradus, J.R., Chettri, P., Forester, N.T. & Mace, W.J. et al. (2021) The dynamic mechanisms underpinning symbiotic *Epichloë*–grass interactions: implications for sustainable and resilient agricultureIn: White, J., Kumar, A. & Droby, S. (Eds.) *Microbiome stimulants for crops*. Woodhead Publishing, pp. 73–108.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S. et al. (2012) Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, 28, 1647–1649.

Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, 35, 1547–1549.

Leeuw, J.D. & Mair, P. (2015) Shepard diagram. In: Balakrishnan, N., Colton, T., Everitt, B., Piegorsch, W., Ruggeri, F. & Teugels, J.L. (Eds.) *Wiley StatsRef: Statistics reference online*. American Cancer Society, pp. 1–3.

Lind, E.M., Borer, E., Seabloom, E., Adler, P., Bakker, J.D., Blumenthal, D.M. et al. (2013) Life-history constraints in grassland plant species: a growth-defence trade-off is the norm. *Ecology Letters*, 16, 513–521.

Li, S., Lin, D., Zhang, Y., Deng, M., Chen, Y. & Lv, B. et al. (2022) Genome-edited powdery mildew resistance in wheat without growth penalties. *Nature*, 602, 455–460.

Liu, B., Ju, Y., Xia, C., Zhong, R., Christensen, M.J., Zhang, X. et al. (2022) The effect of *Epichloë* endophyte on phyllosphere microbes and leaf metabolites in *Achnatherum inebrians*. *iScience*, 25, 104144.

Liu, B., Seong, K., Pang, S., Song, J., Gao, H., Wang, C. et al. (2021) Functional specificity, diversity, and redundancy of *Arabidopsis* JAZ family repressors in jasmonate and COI1-regulated growth, development, and defense. *New Phytologist*, 231, 1525–1545.

Liu, H., Tang, H., Ni, X., Zhang, J. & Zhang, X. (2022) *Epichloë* endophyte interacts with saline-alkali stress to alter root phosphorus-solubilizing fungal and bacterial communities in tall fescue. *Frontiers in Microbiology*, 13, 1027428.

Liu, Q., Parsons, A.J., Xue, H., Fraser, K., Ryan, G.D., Newman, J.A. et al. (2011) Competition between foliar *Neotyphodium lolii* endophytes and mycorrhizal *Glomus* spp. fungi in *Lolium perenne* depends on resource supply and host carbohydrate content. *Functional Ecology*, 25, 910–920.

Li, Y., Yang, Y., Hu, Y., Liu, H., He, M., Yang, Z. et al. (2019) DELLA and EDS1 form a feedback regulatory module to fine-tune plant growth–defense tradeoff in *Arabidopsis*. *Molecular Plant*, 12, 1485–1498.

Love, M.I., Huber, W. & Anders, S. (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq. 2. *Genome Biology*, 15, 550.

Major, I.T., Guo, Q., Zhai, J., Kapali, G., Kramer, D.M. & Howe, G.A. (2020) A phytochrome B-independent pathway restricts growth at high levels of jasmonate defense. *Plant Physiology*, 183, 733–749.

Mathew, S.A., Helander, M., Saikkonen, K., Vankova, R., Dobrev, P.I., Dirihan, S. et al. (2023) *Epichloë* endophytes shape the foliar endophytic fungal microbiome and alter the auxin and salicylic acid phytohormone levels in two meadow fescue cultivars. *Journal of Fungi*, 9, 90.

May, K.J., Bryant, M.K., Zhang, X., Ambrose, B. & Scott, B. (2008) Patterns of expression of a lolitrem biosynthetic gene in the *Epichloë festucae*-perennial ryegrass symbiosis. *Molecular Plant-Microbe Interactions®*, 21, 188–197.

Miller, T.A., Hudson, D.A., Johnson, R.D., Singh, J.S., Mace, W.J., Forester, N.T. et al. (2022) Dissection of the epoxyjanthitrem pathway in *Epichloë* sp. LpTG-3 strain AR37 by CRISPR gene editing. *Frontiers in Fungal Biology*, 3, 944234.

Monson, R.K., Trowbridge, A.M., Lindroth, R.L. & Lerdau, M.T. (2022) Coordinated resource allocation to plant growth–defense tradeoffs. *New Phytologist*, 233, 1051–1066.

Morella, N.M., Weng, F.C.-H., Joubert, P.M., Metcalf, C.J.E., Lindow, S. & Koskella, B. (2020) Successive passaging of a plant-associated microbiome reveals robust habitat and host genotype-dependent selection. *Proceedings of the National Academy of Sciences*, 117, 1148–1159.

Navarro, L., Bari, R., Achard, P., Lisón, P., Nemri, A., Harberd, N.P. et al. (2008) DELLAs control plant immune responses by modulating the balance of jasmonic acid and salicylic acid signaling. *Current Biology*, 18, 650–655.

Nissinen, R., Helander, M., Kumar, M. & Saikkonen, K. (2019) Heritable *Epichloë* symbiosis shapes fungal but not bacterial communities of plant leaves. *Scientific Reports*, 9, 5253.

Noorifar, N., Savoian, M.S., Ram, A., Lukito, Y., Hassing, B., Weikert, T.W. et al. (2021) Chitin deacetylases are required for *Epichloë festucae* endophytic cell wall remodeling during establishment of a mutualistic symbiotic interaction with *Lolium perenne*. *Molecular Plant-Microbe Interactions®*, 34, 1181–1192.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P. & McGlinn, D. et al. (2020) *Vegan: community ecology package*. R package, Version 2.5-7.

Panda, S., Jozwiak, A., Sonawane, P.D., Szymanski, J., Kazachkova, Y., Vainer, A. et al. (2022) Steroidal alkaloids defence metabolism and plant growth are modulated by the joint action of gibberellin and jasmonate signalling. *New Phytologist*, 233, 1220–1237.

Pangesti, N., Pineda, A., Pieterse, C.M.J., Dicke, M. & Van Loon, J.J.A. (2013) Two-way plant mediated interactions between root-associated microbes and insects: from ecology to mechanisms. *Frontiers in Plant Science*, 4, 414.

Peng, Y., Yang, J., Li, X. & Zhang, Y. (2021) Salicylic acid: biosynthesis and signaling. *Annual Review of Plant Biology*, 72, 761–791.

Pérez, L.I., Gundel, P.E., Ghersa, C.M. & Omacini, M. (2013) Family issues: fungal endophyte protects host grass from the closely related pathogen *Claviceps purpurea*. *Fungal Ecology*, 6, 379–386.

Pfeilmeier, S., Petti, G.C., Bortfeld-Miller, M., Daniel, B., Field, C.M., Sunagawa, S. et al. (2021) The plant NADPH oxidase RBOHD is required for microbiota homeostasis in leaves. *Nature Microbiology*, 6, 852–864.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. & Core Team, R. (2009) *nlme: linear and nonlinear mixed effects models*. R package.

R Core Team. (2022) R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/

Rasmussen, S., Parsons, A.J., Bassett, S., Christensen, M.J., Hume, D.E., Johnson, L.J. et al. (2007) High nitrogen supply and carbohydrate content reduce fungal endophyte and alkaloid concentration in *Lolium perenne*. *New Phytologist*, 173, 787–797.

Rasmussen, S., Parsons, A.J., Russell, J., Bastías, D.A. & Liu, Q. (2023) Plant species, nitrogen status and endophytes are drivers of soil microbial communities in grasslands. *Crop and Pasture Science*, 75, CP23149.

Roberts, E. & Lindow, S. (2014) Loline alkaloid production by fungal endophytes of fescue species select for particular epiphytic bacterial microflora. *The ISME journal*, 8, 359–368.

Robert-Seilaniantz, A., Grant, M. & Jones, J.D.G. (2011) Hormone crosstalk in plant disease and defense: more than just JASMONATE-SALICYLATE antagonism. *Annual Review of Phytopathology*, 49, 317–343.

Santner, A., Calderon-Villalobos, L.I.A. & Estelle, M. (2009) Plant hormones are versatile chemical regulators of plant growth. *Nature Chemical Biology*, 5, 301–307.

Sauret-Güeto, S., Calder, G. & Harberd, N.P. (2012) Transient gibberellin application promotes *Arabidopsis thaliana* hypocotyl cell elongation without maintaining transverse orientation of microtubules on the outer tangential wall of epidermal cells. *The Plant Journal*, 69, 628–639.

Schardl, C.L., Leuchtmann, A. & Spiering, M.J. (2004) Symbioses of grasses with seedborne fungal endophytes. *Annual Review of Plant Biology*, 55, 315–340.

Schmid, J., Day, R., Zhang, N., Dupont, P.-Y., Cox, M.P., Schardl, C.L. et al. (2017) Host tissue environment directs activities of an *Epichloë* endophyte, while it induces systemic hormone and defense responses in its native perennial ryegrass host. *Molecular Plant-Microbe Interactions®*, 30, 138–149.

Scott, B., Green, K. & Berry, D. (2018) The fine balance between mutualism and antagonism in the *Epichloë festucae*–grass symbiotic interaction. *Current Opinion in Plant Biology*, 44, 32–38.

Sestari, I. & Campos, M.L. (2022) Into a dilemma of plants: the antagonism between chemical defenses and growth. *Plant Molecular Biology*, 109, 469–482.

Simpson, W.R., Schmid, J., Singh, J., Faville, M.J. & Johnson, R.D. (2012) A morphological change in the fungal symbiont *Neotyphodium lolii* induces dwarfing in its host plant *Lolium perenne*. *Fungal Biology*, 116, 234–240.

Stant, M.Y. (1963) The effect of gibberellic acid on cell width and the cell-wall of some phloem fibres. *Annals of Botany*, 27, 185–196.

Sullivan, T.J., Rodstrom, J., Vandop, J., Librizzi, J., Graham, C., Schardl, C.L. et al. (2007) Symbiont-mediated changes in *Lolium arundinaceum* inducible defenses: evidence from changes in gene expression and leaf composition. *New Phytologist*, 176, 673–679.

Tang, J., Wu, D., Li, X., Wang, L., Xu, L., Zhang, Y. et al. (2022) Plant immunity suppression via PHR1-RALF-FERONIA shapes the root microbiome to alleviate phosphate starvation. *The EMBO Journal*, 41, e109102.

Thaler, J.S., Humphrey, P.T. & Whiteman, N.K. (2012) Evolution of jasmonate and salicylate signal crosstalk. *Trends in Plant Science*, 17, 260–270.

De Vleesschauwer, D., Seifi, H.S., Filipe, O., Haeck, A., Huu, S.N., Demeestere, K. et al. (2016) The DELLA protein SLR1 integrates and amplifies salicylic acid-and jasmonic acid-dependent innate immunity in rice. *Plant Physiology*, 170, 1831–1847.

Voisey, C.R. (2010) Intercalary growth in hyphae of filamentous fungi. *Fungal Biology Reviews*, 24, 123–131.

Wang, Q., Garrity, G.M., Tiedje, J.M. & Cole, J.R. (2007) Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology*, 73, 5261–5267.

Wooley, J.C., Godzik, A. & Friedberg, I. (2010) A primer on metagenomics. *PLoS Computational Biology*, 6, e1000667.

Xia, G., Wu, C., Zhang, M., Yang, F., Chen, C. & Hao, J. (2023) The metabolome and bacterial composition of high-moisture Italian ryegrass silage inoculated with lactic acid bacteria during ensiling. *Biotechnology for Biofuels and Bioproducts*, 16, 91.

Xie, X.-G., Lu, W.-L., Feng, K.-M., Zheng, C.-J., Yang, Y., Jia, M. et al. (2023) Mechanisms of *Epichloë bromicola* to promote plant growth and its potential application for *Coix lacryma-jobi* L. cultivation. *Current Microbiology*, 80, 306.

Yue, Q., Miller, C.J., White, J.F. & Richardson, M.D. (2000) Isolation and characterization of fungal inhibitors from *Epichloë festucae*. *Journal of Agricultural and Food Chemistry*, 48, 4687–4692.

Zhang, D.X., Nagabhyru, P. & Schardl, C.L. (2009) Regulation of a chemical defense against herbivory produced by symbiotic fungi in grass plants. *Plant Physiology*, 150, 1072–1082.

Zhang, W., Forester, N.T., Applegate, E.R., Liu, X. & Johnson, L.J. (2023) High-affinity iron uptake is required for optimal *Epichloë festucae* colonization of *Lolium perenne* and seed transmission. *Molecular Plant Pathology*, 24, 1430–1442.

Zhang, W., Mace, W.J., Matthew, C. & Card, S.D. (2019) The impact of endophyte infection, seed aging, and imbibition on selected sugar metabolite concentrations in seed. *Journal of Agricultural and Food Chemistry*, 67, 6921–6929.

Zhong, R., Xia, C., Ju, Y., Li, N., Zhang, X., Nan, Z. et al. (2018) Effects of *Epichloë gansuensis* on root-associated fungal communities of *Achnatherum inebrians* under different growth conditions. *Fungal Ecology*, 31, 29–36.

Züst, T. & Agrawal, A.A. (2017) Trade-offs between plant growth and defense against insect herbivory: an emerging mechanistic synthesis. *Annual Review of Plant Biology*, 68, 513–534.

Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A. & Smit, G.M. (2009) *Mixed effects models and extensions in ecology with R*, 1st ed. Springer Sciences.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Zhang, W., Gundel, P.E., Jáuregui, R., Card, S.D., Mace, W.J., Johnson, R.D. et al. (2024) The growth promotion in endophyte symbiotic plants does not penalise the resistance to herbivores and bacterial microbiota. *Plant, Cell & Environment*, 2865–2878. https://doi.org/10.1111/pce.14912