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# Environmental conditions and plant diversity show little effect on mycotoxin occurrence in European grasslands used for horse husbandry

## Umweltbedingungen und Pflanzendiversität haben wenig Einfluss auf das Auftreten von Mykotoxinen in europäischen Grünlandflächen für die Pferdehaltung

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**Abstract**

In light of ongoing threats to European grasslands, grazing by livestock such as horses can contribute to biodiversity conservation. An emerging field of research relevant to both grassland ecosystems and animal husbandry has focused on grass-endophyte symbioses between *Epichloe* fungi and grasses of the genera *Festuca, Lolium,* and *Schedonorus*. These endophytes produce toxic alkaloids and have been associated with certain fitness benefits to host grasses, possibly leading to diminished grassland diversity. However, these grass-endophyte symbioses have rarely been studied in (semi-) natural settings. In this study we explored the prevalence of mycotoxins produced by fungal endophytes in temperate European grasslands, testing for possible links between mycotoxin occurrence and ecological conditions or land use.

We sampled 310 vegetation plots of 10 $m^2$ at seven horse sanctuaries ("sites") in France, Germany, Austria, and Hungary. In a subset of 204 plots, we collected and tested 372 samples of the target grass genera for the mycotoxins ergovaline and lolitrem B using ultra-performance liquid chromatography–mass spectrometry (UPLC-MS). For all plots, we calculated plant diversity measures and mean Ecological Indicator Values for Europe (EIVEs). Differences in the vegetation and mycotoxin prevalence were tested using linear mixed-effects models. To explore ecological effects on mycotoxin prevalence, we calculated generalized mixed models at plot and sample level.

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Plant diversity was similar to averages for the countries studied, with maxima and Red List species occurring in dryer and wetter conditions. Mycotoxins occurred at all sites under a broad range of environmental conditions. Mycotoxin occurrence was comparable to endophyte infection rates from the literature. *Festuca rubra* aggr. tested positive at rates exceeding twice that of *Lolium perenne* and *Schedonorus arundinaceus*. Infection was associated with higher soil moisture and lower nitrogen EIVEs, in contrast to the literature showing benefits to hosts primarily in dry and nutrient-rich conditions. Grass cover and host dominance showed contrasting effects on mycotoxin prevalence in host species. There was no relationship between mycotoxin prevalence and temperature, biodiversity, or land-use type.

From our results, we conclude that endophyte infection is common in semi-natural grasslands in temperate Europe but appears to currently present only a limited risk to livestock. We find no strong or consistent relationships between mycotoxin occurrence and ecological conditions or plant diversity. This is consistent with evidence that the effects of infection on hosts are context-dependent and complex. Species-rich grasslands may provide a protective effect against endophyte toxicosis in livestock by diluting mycotoxin concentrations where endophyte infection occurs.

**Keywords**: biodiversity, *Epichloe*, *Festuca rubra* aggr., fungal endophyte, *Lolium perenne*, meadow, semi-natural grasslands, toxin, horse pasture

**Erweiterte deutsche Zusammenfassung am Ende des Artikels**

## 1. Introduction

Semi-natural grasslands support remarkable biodiversity (Dengler et al. 2014) and provide vital ecosystem services (World Resources Institute 2005, Bengtsson et al. 2019), but are threatened both by land-use intensification and abandonment throughout temperate Europe (Török & Dengler 2018, Valkó et al. 2018). In light of these threats, low-intensity grazing by horses can contribute to grassland biodiversity conservation (e.g. Bokdam et al. 2002, Köhler et al. 2016, Henning et al. 2017). Various elements of horse physiology and behavior make them of particular interest for sustainable grassland management, with traditional horse husbandry practices such as late grazing and mowing, grazing low-productivity sites, and use of forested pastureland offering the potential to conserve and restore grassland biodiversity (Chodkiewicz 2020, Vanselow 2021). However, general studies on the ecological effects of horse grazing are thus far rare.

Due to their wide-ranging effects on grasses and livestock, grass endophytes are relevant to both horse husbandry and grassland ecosystem functioning. These endophytes include fungi of the epichloe clade (*Epichloe* s.l. including former *Neotyphodium* species according to Leuchtmann et al. 2014; hereafter "endophytes") which are systemic, often asymptomatic symbionts of grasses that produce chemically diverse toxic alkaloids which deter insect and mammalian herbivory (Clay 1988, Schardl et al. 2012). Grass-endophyte symbioses are thought to be associated with certain benefits to hosts, including enhanced vigor and resistance to environmental stress such as drought (Bacon & White 2000, Cheplick & Faeth 2009). These symbioses have also been shown to negatively influence plant biodiversity in grasslands, possibly due to the increased competitive ability of infected plants (Clay & Holah 1999, Malinowski & Belesky 2006). Grass-endophyte relationships appear to be affected by both biotic factors such as herbivory and abiotic factors such as temperature and water availability (e.g. Cheplick & Faeth 2009), although our understanding of this complex system is still incomplete. Most studies on grass-endophyte ecology have taken place within nutrient-rich agricultural or greenhouse settings and have not focused on grasslands under natural environmental conditions (Saikkonen et al. 2006, König et al. 2018, Leinonen et al.

2019). Additionally, since much of the research on grass-endophyte symbioses has focused on *Lolium perenne* and *Schedonorus arundinaceus* and was conducted in regions where these species are not native (i.e. in North America and New Zealand), knowledge on the ecology of their endophyte associations in their native range is lacking.

Thus, with this study, we aimed to improve the state of knowledge on the endophyte-grassland-grazer system by following two objectives: (1) Exploring the prevalence of endophytes in temperate European meadows and pastures used for horse husbandry by means of associative mycotoxin analysis; and (2) Investigating environmental conditions and patterns of biodiversity in such meadows and pastures, and searching for possible links to endophyte occurrence.

## 2. Study area

The study area lies between 46.63141°–49.28694° N and 3.33810°–17.79541° E (WGS 84), spanning four temperate European countries: France, Germany, Austria, and Hungary (Fig. 1). Within these countries, the seven study sites are part of a network of associated animal sanctuaries where horses are kept (Table 1); there are no commercial agricultural activities at the sites, nor are the animals used for sport or as working animals.

The studied grasslands cover an elevational gradient from approximately 250 to 650 m a.s.l., with the lowest elevations in France and the highest in Austria. The grasslands are mostly mesic and nutrient rich, although a few are semidry or wet. At the time of sampling, they were used as horse pastures or as meadows where hay is harvested for horses (Fig. 2). According to the phytosociological typology of Mucina et al. (2016), the large majority of the grasslands belonged to the class *Molinio-Arrhenatheretea* Tx. 1937, mostly the order *Arrhenatheretalia elatioris* Tx. 1931 with the alliances *Arrhentherion elatioris* Luquet 1926 and *Cynosurion cristati* Tx. 1947, partly also the order *Molinietalia caeruleae* Koch 1926 with the alliance *Calthion palustris* Tx. 1937. Smaller parts of the plots belonged

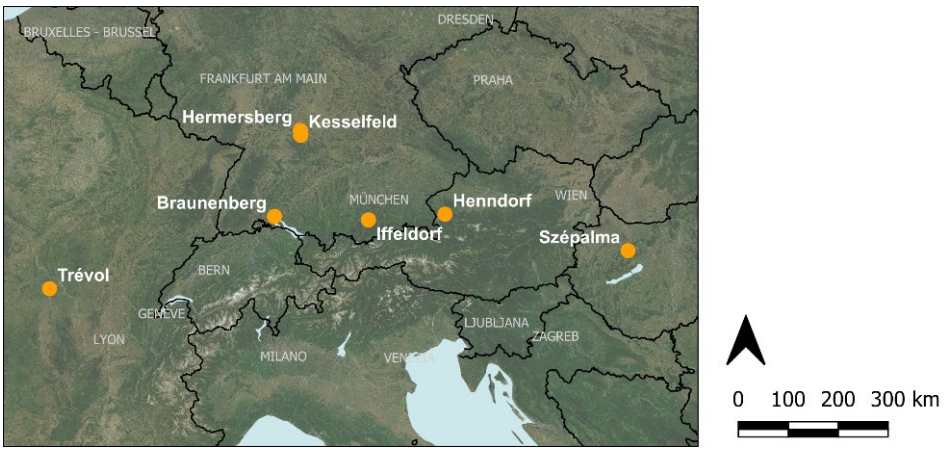


**Fig. 1.** Map of the study sites in Europe. Hermersberg and Kesselfeld are located very close together (geodata: OpenStreetMap contributors, available from https://www.openstreetmap.org).

**Abb. 1.** Karte der Untersuchungsgebiete in Europa. Hermersberg und Kesselfeld liegen sehr nahe beieinander (Geodaten: OpenStreetMap-Mitwirkende, verfügbar unter https://www.openstreetmap.org).

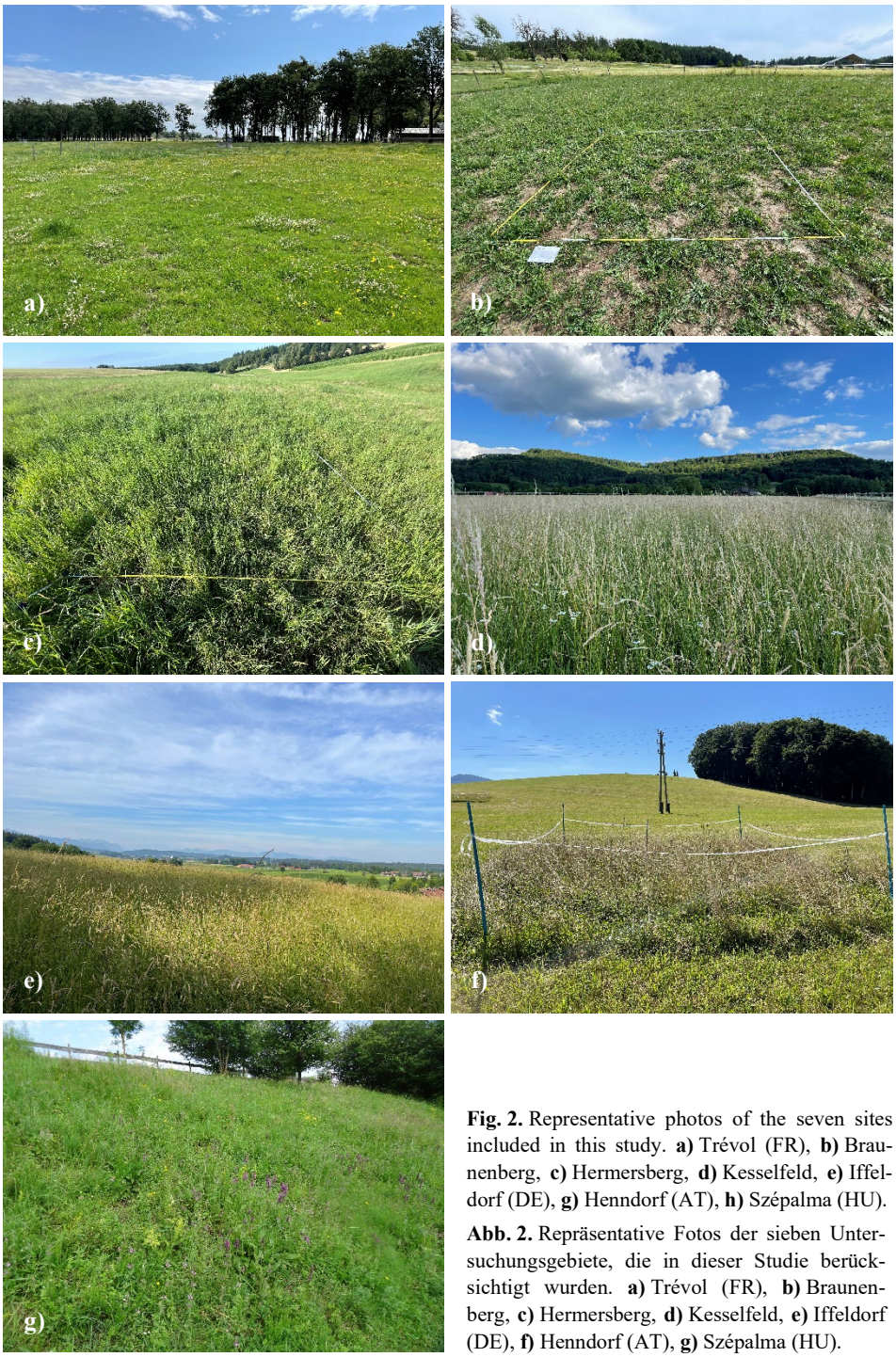


**Fig. 2.** Representative photos of the seven sites included in this study. **a)** Trévol (FR), **b)** Braunenberg, **c)** Hermersberg, **d)** Kesselfeld, **e)** Iffeldorf (DE), **g)** Henndorf (AT), **h)** Szépalma (HU).

**Abb. 2.** Repräsentative Fotos der sieben Untersuchungsgebiete, die in dieser Studie berücksichtigt wurden. **a)** Trévol (FR), **b)** Braunenberg, **c)** Hermersberg, **d)** Kesselfeld, **e)** Iffeldorf (DE), **f)** Henndorf (AT), **g)** Szépalma (HU).

**Table 1.** Geographical and climatic information for the seven study sites, arranged from west to east. Site "size" includes all land use types, including buildings and other infrastructure. Grasslands consist of fenced or otherwise demarcated meadows and pastures. Climate data: CHELSA V2.1 (Chelsa Climate 2025, Karger et al. 2017).

**Tabelle 1.** Geografische und klimatische Angaben für die sieben Untersuchungsgebiete, angeordnet von West nach Ost. Die „Größe" der Gebiete umfasst alle Landnutzungsarten, einschließlich Gebäude und anderer Infrastruktur. Grasland besteht aus eingezäunten oder anderweitig abgegrenzten Wiesen und Weiden. Klimadaten: CHELSA V2.1 (Chelsa Climate 2025, Karger et al. 2017).

| Site | Acronym | Administrative region (country) | Size (ha) | Number of grasslands surveyed (% pastures) | Mean annual temperature (°C) | Mean annual precipitation (mm) |
|---|---|---|---|---|---|---|
| Trévol | TR | Allier (FR) | 120 | 11 (82%) | 11.6 | 815 |
| Braunenberg | BB | Baden-Württemberg (DE) | 100 | 5 (60%) | 9.1 | 873 |
| Hermersberg | HB | Baden-Württemberg (DE) | 80 | 5 (40%) | 9.3 | 876 |
| Kesselfeld | KF | Baden-Württemberg (DE) | 40 | 5 (0%) | 9.2 | 914 |
| Iffeldorf | ID | Bavaria (DE) | 150 | 10 (40%) | 8.4 | 1293 |
| Henndorf | HD | Salzburg (AT) | 140 | 10 (40%) | 7.3 | 1437 |
| Szépalma | SP | Veszprém (HU) | 130 | 10 (60%) | 8.7 | 767 |

to the *Festuco-Brometea* Br.-Bl. et Tx. ex Soó 1947 (*Brachypodietalia pinnati* Korneck 1974 nom. conserv. propos.: *Cirsio-Brachypodion pinnati* Hadač et Klika in Klika et Hadač 1944 – in Hungary), *Scheuchzerio palustris-Caricetea fuscae* Tx. 1937, *Phragmito-Magnocaricetea* Klika in Klika et Novák 1941 and *Polygono-Poetea annuae* Rivas-Martínez 1975. Within each grassland, we sampled a selection of vegetation plots ("plots"; Section 3.1).

# 3. Materials and methods

## 3.1 Field sampling

We carried out the sampling from 2021 to 2024 at seven sites (Fig. 1, Table 1) during a series of four individual projects. Sampling occurred from June to August, except in Trévol, where we sampled in late April and May to avoid the exceptionally dry summers. Due to the strongly local and practical nature of the original surveys and their project aims, the selection of grasslands and plot locations did not follow a uniform scheme. Sampling schemes (grassland selection and plot placement) ranged from stratified-systematic to random. The aims of the individual local projects included surveying grasslands where endophyte infection was suspected, documenting areas of low to high ecological value, and providing baseline information for a pasture restoration project. However, all plots were sampled using the same method.

Per site, we surveyed 5–11 grasslands, with five plots per grassland (except six grasslands in France, which were sampled with ten plots each) (Table 1). Each grassland and plot was sampled only once during the four-year period. Grasslands were roughly evenly divided between meadows ($n$ = 140) and pastures ($n$ = 170), although sites differed in the proportion of management types sampled (all of them ranged from 40–60% meadow, except for Kesselfeld with exclusively meadow and Trévol with 82% pasture). This is because management type was not a systematically investigated factor in the original projects. In the few cases where grasslands were both grazed and mown, plots were assigned to the "pasture" category.

The vegetation data consist of 310 precisely delimited, square-shaped plots of 10 $m^2$. In each plot, we visually estimated the percent cover of each vascular plant found within the plot (see Dengler & Dembicz 2023, Dembicz & Dengler 2025) as well as total cover of vegetation layers (bryophyte, herbaceous, shrub, and tree), litter, and stones. We recorded aspect and inclination in degrees and noted the GPS coordinates. In France, Germany, and Austria, plot data were recorded using FlorApp, a smartphone application developed by the National Data and Information Center on the Swiss Flora (InfoFlora 2025a). This application provides a tool for collecting georeferenced and timestamped vegetation plot data and managing them over an online platform, the Info Flora field book (InfoFlora 2025b). We used regional floras to identify vascular plants, and the national Red Lists to determine their conservation status (France – UICN France et al. 2018, Germany – Metzing et al. 2018, Austria – Schratt-Ehrendorfer et al. 2022, Hungary – Király 2007). Nomenclature corresponds to the Euro+Med taxonomy (Euro+Med 2025). The vegetation-plot data are available from the authors upon request.

In each plot, we collected samples of each species of the genera *Festuca*, *Lolium*, and *Schedonorus* present with at least 1% cover within the plot. These genera are widespread in nutrient-rich temperate grasslands and include globally important forage crops which are known to be common hosts of *Epichloe* (Cheplick & Faeth 2009). Samples consisted of ca. 20–30 g of plant material comprising parts known to contain elevated mycotoxin concentrations (inflorescences, seeds, and basal plant parts including lower leaf sheaths) (Spiering et al. 2005). The phenological stage of each sample (Meier 2001) was noted. If the volume of material within the plot was insufficient, we collected additional sample material within 1–2 m around the plot. Samples were stored in paper bags and allowed to air dry at room temperature before being sent to the lab.

### 3.2 Mycotoxin analysis

Mycotoxin testing was carried out in November or December following each sampling campaign. Due to differing aims within the original projects, not all grass samples were tested for mycotoxins. 372 samples collected within a selection of 204 plots (Supplement E1) were tested for the endophyte alkaloids lolitrem B and ergovaline using ultra-performance liquid chromatography–mass spectrometry (UPLC-MS) (lab protocol: Supplement E2). These mycotoxins are known to be produced by *Epichloe* in the host taxa and were selected due to their known toxic effects on livestock and their correlation with the presence of endophyte mycelium (Spiering et al. 2005). These analyses resulted in two types of data: binomial data indicating the presence or absence of mycotoxins, and measurements of the concentration of individual mycotoxins within the samples. To account for the wide variability in mycotoxin concentrations which has shown to be independent from endophyte performance (Spiering et al. 2005, Fuchs et al. 2013, Repussard et al. 2014), we included only the binomial data in our analyses and present the mycotoxin concentrations only as supporting information.

### 3.3 Data analysis

Vegetation data were managed in VEGEDAZ (Küchler 2024) and the InfoFlora field book (InfoFlora 2025b). All analyses were performed at plot level unless otherwise specified. We used VEGEDAZ to calculate biodiversity metrics (species richness, Shannon index, Shannon evenness). We performed all further statistical analyses using R v. 4.3.0 (R Core Team 2023). For statistical testing, we employed a significance threshold of $\alpha = 0.05$. Where appropriate, we checked for model validity by inspecting model residuals visually.

We used R to calculate cover-weighted ecological indicator values (scale 0–10) on the basis of the pan-European system EIVE v. 1.0 (Dengler et al. 2023; for relative performance compared to other indicator value systems, see Ostrowski et al. 2025). EIVE comprises the climatic factors of light availability and temperature, and the edaphic factors of soil reaction, soil moisture, and soil nitrogen content. These values were calculated at plot level for the complete vegetation (i.e. including host plants).

To test for the differences between sites, we calculated analysis of variance with grassland as an error term. After excluding the two sites with extremely unbalanced sampling of management types (Kesselfeld and Trévol), ecological and diversity differences between pastures and meadows were

tested using mixed models with grassland and site as nested random factors. The models were calculated using the *lmer* command from the R package "lmerTest" (Kuznetsova et al. 2017), and took the following structure:

*lmer(response_variable~land_use + (1| site/grassland), data = header)*

To visualize overall trends in the floristic data, we calculated an ordination of the plots using detrended correspondence analysis (DCA) with the *decorana* command from the R package "vegan" (Oksanen et al. 2025). The use of DCA was justified by the gradient length of the first axis (Lepš & Šmilauer 2003). Using the *envfit* command from the same package, we calculated a multiple regression of the EIVEs with ordination axes and projected them post hoc onto the ordination plot to aid in interpretation.

To explore possible links between ecological conditions and mycotoxin prevalence, we calculated a series of generalized mixed models. Predictor variables were related to biodiversity (species richness, Shannon index, Shannon evenness), abiotic conditions (soil moisture EIVE, soil nitrogen EIVE, temperature EIVE), and land use (mowing vs. grazing). For the 44 plots with complete testing (i.e. all *Festuca*, *Lolium*, and *Schedonorus* species present within the plot were sampled and tested), we used logistic regressions to determine if ecological conditions or biodiversity metrics affected endophyte (i.e. mycotoxin) occurrence in all tested grasses within the plots. At grass sample level, we again used logistic regressions to determine if ecological conditions or biodiversity metrics affected mycotoxin occurrence within the two species with sample size > 100 which tested positive most frequently (*Festuca rubra* aggr., 108 samples, and *Lolium perenne*, 141 samples). To this end, we tested the presence/absence of mycotoxins as a binomial response variable using generalized mixed models with a random structure of grasslands nested within sites. Land use was not included as a second fixed factor, since tests determined it did not have any significant association with the ecological conditions which are hypothesized to influence endophyte infection. The models were calculated using the *glmer* command from the R package "lme4" (Bates et al. 2015), and took the following structure:

*glmer(mycotoxin_presence~predictor_variable + (1|site/grassland), family = binomial, data = dat)*

All models were calculated with aggregated mycotoxin occurrence, as well as separately for the two mycotoxins (ergovaline and lolitrem B). If random factors were shown to explain very little variance (boundary warning in R), they were excluded stepwise from the model, with nested effects excluded first.

# 4. Results

## 4.1 Biodiversity

The mean vascular plant species richness was 25.1 in 10 $m^2$, while the maximum was 48 species in 10 $m^2$. Average species richness varied significantly between sites ($p = 0.011$), but individual sites showed broad ranges in species richness (Fig. 3). No significant relationship between biodiversity metrics and land use (mowing vs. grazing) could be detected (Table 2). The highest species richness was found in semidry low-intensity pastures in Szépalma (HU), while the most species-poor grassland was a productive meadow in Braunenberg (DE; Fig. 4). In total, eight species of the national Red Lists were found at four different sites (Supplement E3).

## 4.2 Ecological conditions

Most ecological conditions varied significantly between sites (Table 2), but effect sizes were small. Variation within sites was usually modest (Fig. 5). Differences in temperature EIVEs among sites were significant, but this was the least variable of the tested EIVEs

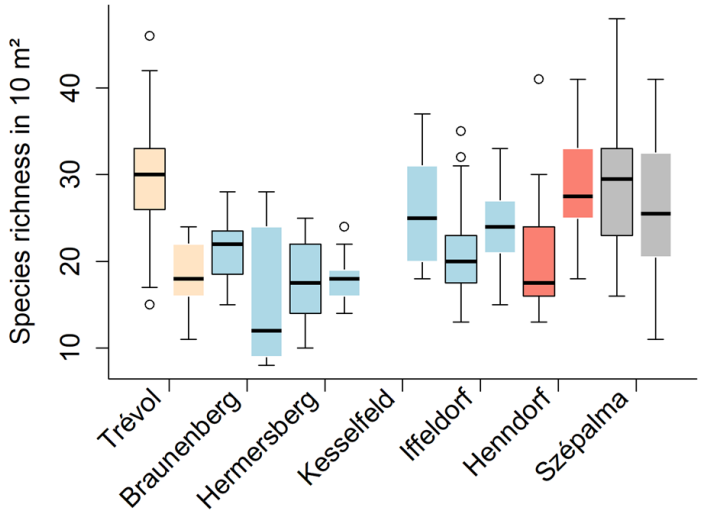


**Fig. 3.** Species richness between sites and land-use types, color coded by country. Beige – France; blue – Germany; red – Austria; grey – Hungary. Outlined boxes – pastures; boxes without outlines – meadows.

**Abb. 3.** Artenreichtum nach Untersuchungsgebiet und Landnutzungstyp, farblich nach Ländern gekennzeichnet. Beige – Frankreich; blau – Deutschland; rot – Österreich; grau – Ungarn. Umrandete Boxen – Weiden; Boxen ohne Umrandung – Wiesen.


**Fig. 4.** The most species poor plot was in productive hay meadow in Braunenberg (**a)** BB-5, with 8 species in 10 $m^2$), while the most species rich was in a semidry pasture in Szépalma (**b)** HU-8, with 48 species in 10 $m^2$).

**Abb. 4.** Die artenärmste Aufnahme befand sich auf einer produktiven Heuwiese in Braunenberg (**a)** BB-5, mit 8 Arten auf 10 $m^2$), während die artenreichste Aufnahme auf einer halbtrockenen Weide in Szépalma (**b)** HU-8, mit 48 Arten auf 10 $m^2$) lag.

**Table 2.** Descriptive statistics and model results for square-root cover-weighted ecological indicator values (EIVEs, range 0–10), covers of species groups and biodiversity metrics. Differences among sites were tested using analysis of variance with grassland as error term, while differences between land-use types (mowing vs. grazing) were tested using linear mixed models with grassland and site as nested random effects. Min. – minimum value, Max. – maximum value, SD – standard deviation. Significance levels: * – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$.

**Tabelle 2.** Deskriptive Statistik und Modellergebnisse für quadratwurzelgewichtete ökologische Indikatorwerte (EIVEs, Skala 0–10), Deckungswerte und Biodiversitätsmetriken. Die Unterschiede zwischen den Standorten wurden mittels Varianzanalyse mit Grasland als Fehlerterm getestet, während die Unterschiede zwischen den Landnutzungstypen (Wiesen vs. Weiden) mittels linearer gemischter Modelle mit Grasland und Untersuchungsort als verschachtelten Zufallsfaktoren getestet wurden. Min. – Minimalwert, Max. – Maximalwert, SD – Standardabweichung. Signifikanzniveaus: * – $p < 0{,}05$; ** – $p < 0{,}01$; *** – $p < 0{,}001$.

| | Mean | SD | *p*, sites | | *p*, land-use type |
|---|---|---|---|---|---|
| Temperature EIVE | 4.14 | 0.14 | **0.005** | ** | 0.779 |
| Light EIVE | 7.14 | 0.24 | 0.161 | | 0.930 |
| Soil moisture EIVE | 4.53 | 0.33 | **< 0.001** | *** | 0.154 |
| Soil reaction EIVE | 5.82 | 0.36 | **< 0.001** | *** | 0.058 |
| Soil nitrogen EIVE | 5.36 | 0.68 | 0.002 | ** | 0.811 |
| Poaceae cover (%) | 60.9 | 18.5 | 0.277 | | 0.071 |
| *Lolium, Festuca,* and *Schedonorus* cover (%) | 22.7 | 14.4 | 0.273 | | 0.820 |
| Species richness in 10 $m^2$ | 25.1 | 7.4 | **0.011** | * | 0.365 |
| Shannon index | 2.81 | 0.33 | 0.220 | | 0.308 |
| Shannon evenness | 0.89 | 0.04 | 0.080 | | 0.067 |

overall (SD = 0.14 among all plots; Fig. 5a). Water availability differed but was mostly within the middle of the gradient, with higher values in Iffeldorf and Henndorf and lower ones in Trévol and Szépalma (Fig. 5b). Soils were more acidic in Trévol and Szépalma than at the other sites (Fig. 5c). The sites were predominantly nutrient rich but varied in nutrient availability, with nitrogen EIVEs being the most variable of all EIVEs (SD = 0.68; Fig. 5d). Land use (mowing vs. grazing) did not have a significant effect on any of the tested response variables (Table 2).

While plotting the DCA, we chose the axis combination which achieved the most easily interpretable spread of data (Fig. 6). DCA axes 1 and 3 explained 27% and 24% of the variance of the floristic data, respectively. Both axes had a gradient length of over 4 standard deviations, justifying the use of DCA (Lepš & Šmilauer 2003). DCA1 correlated most strongly with nitrogen EIVE ($r = 0.995$), while DCA3 correlated most strongly with soil moisture EIVE ($r = 0.959$) and reaction EIVE ($r = -0.956$). Different countries and land-use types showed no clear separation within the DCA plot.

### 4.3 Mycotoxin occurrence

Poaceae species made up 60.9% of the vegetation cover, on average (Table 2). The common endophyte host genera *Lolium*, *Festuca*, and *Schedonorus* were found in 309 of 310 plots (Table 3), with an average cover of 22.7%. Of these species, *Lolium perenne* and *Schedonorus pratensis* were the most common, occurring in 66.7% and 54.8% of plots, respectively. The commonly studied endophyte host *Schedonorus arundinaceus* was present in only 25.2% of the plots.

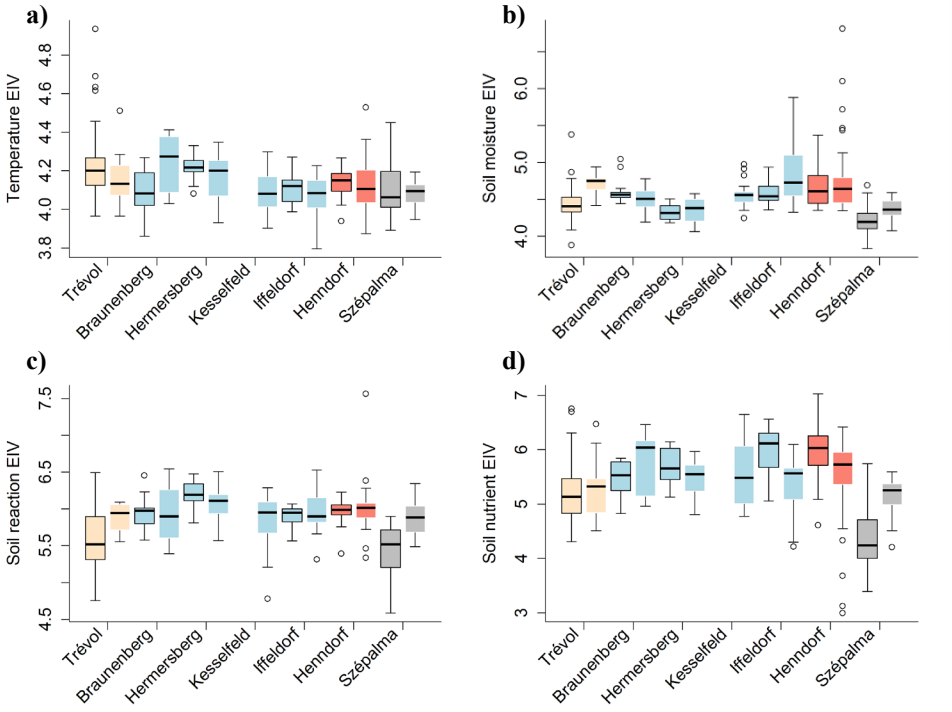


**Fig. 5.** Comparison of mean Ecological Indicator Values for Europe (EIVE; range 0–10) between sites and land-use types, color coded by country. Beige – France; blue – Germany; red – Austria; grey – Hungary. Outlined boxes – pastures; boxes without outlines – meadows.

**Abb. 5.** Vergleich der mittleren ökologischen Indikatorwerte für Europa (EIVE; Skala 0–10) nach Untersuchungsgebiet und Landnutzungstyp, farblich nach Ländern gekennzeichnet. Beige – Frankreich; blau – Deutschland; rot – Österreich; grau – Ungarn. Umrandete Boxen – Weiden; Boxen ohne Umrandung – Wiesen.

Ergovaline and lolitrem B were found in samples from all sites and occasionally reached very high concentrations within the tested samples (Fig. 7). The sites which showed the highest rate of occurrence were Szépalma and Trévol, while Kesselfeld showed barely any mycotoxin occurrence (Supplement E4). Although many plots were not tested completely (i.e. only a selection of *Festuca*, *Lolium*, and *Schedonorus* species were analyzed), mycotoxins were still found in 35.8% of the 204 tested plots (Table 4). Grasses which tested positive for at least one of the mycotoxins were occasionally dominant in the stand: in 11 plots, they made up 25% or more of the vegetation cover.

In 25.5% of the tested samples at least one mycotoxin was detected, while only 4.6% of the samples contained both. *Festuca rubra* aggr. samples tested positive most frequently (45.4%). Other species tested positive much less frequently, with the next highest positivity rates exhibited by *Lolium multiflorum* (25%) and *L. perenne* (24.2%). Out of all the tested species, only in the hybrid *Festulolium ×loliaceum* were no mycotoxins detected ($n = 18$).

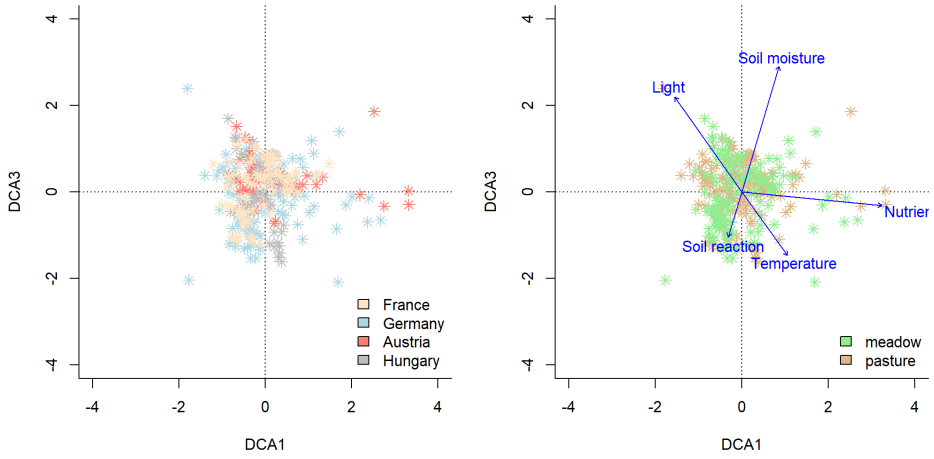


**Fig. 6.** Detrended correspondence analysis (DCA) of the dataset, with plots represented as stars and environmental variables projected onto the ordination. The vectors shown correlate with at least $|r| = 0.80$ with DCA1 or DCA3.

**Abb. 6.** Trendbereinigte Korrespondenzanalyse (DCA) des Datensatzes, mit den Vegetationsaufnahmen als Sterne dargestellt und Umweltvariablen auf die Ordination projiziert. Die dargestellten Vektoren korrelieren mit mindestens $|r| = 0{,}80$ mit DCA1 oder DCA3.

**Table 3.** Descriptive statistics for *Festuca*, *Lolium,* and *Schedonorus* species collected and subject to mycotoxin testing. "Rate of occurrence" refers to positive tests using ultra-performance liquid chromatography–mass spectrometry (UPLC-MS).

**Tabelle 3.** Deskriptive Statistik für *Festuca*-, *Lolium*- und *Schedonorus*-Arten, die gesammelt und auf Mykotoxine getestet wurden. Die „Rate of occurrence" (Häufigkeit des Auftretens) bezieht sich auf positive Tests unter Verwendung von Ultra-Hochleistungsflüssigkeitschromatographie-Massenspektrometrie (UPLC-MS).

| | | | Rate of occurrence (%) | | |
|---|---|---|---|---|---|
| Taxon | # of occurrences | # of samples tested | Total | Ergovaline | Lolitrem B |
| All *Festuca*, *Lolium*, and *Schedonorus* species | 591 | 372 | 25.5 | 17.0 | 13.2 |
| *Festuca heterophylla* | 26 | 0 | - | - | - |
| *Festuca rubra* aggr. | 166 | 108 | 45.4 | 37.0 | 18.5 |
| *Festulolium ×loliaceum* | 18 | 18 | 0 | 0 | 0 |
| *Lolium perenne* | 210 | 141 | 24.1 | 8.5 | 19.1 |
| *Lolium multiflorum* | 27 | 16 | 25.0 | 25.0 | 0.0 |
| *Schedonorus arundinaceus* | 78 | 61 | 11.5 | 9.8 | 1.6 |
| *Schedonorus pratensis* | 170 | 28 | 3.6 | 3.6 | 3.6 |

For the 44 plots with complete testing of all *Festuca*, *Lolium* and *Schedonorus* species, higher soil moisture EIVEs were associated with higher chances of mycotoxin occurrence (estimate 9.4, $p = 0.047$; Table 5). No significant effects could be found for ergovaline occurrence, and there were too few positive tests within these plots to justify models for lolitrem B presence (3 of 44 plots).

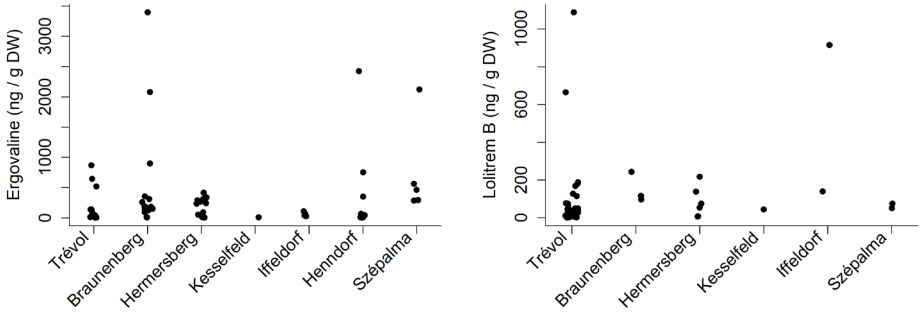


**Fig. 7.** Positive mycotoxin levels in samples of *Festuca*, *Lolium*, and *Schedonorus* species from each site as determined by ultra-performance liquid chromatography–mass spectrometry. No lolitrem B was detected in samples from Henndorf. DW – dry weight.

**Abb. 7.** Mykotoxingehalte in Proben der Gattungen *Festuca*, *Lolium* und *Schedonorus* aus den sieben Untersuchungsgebieten, bestimmt mittels Ultra-Hochleistungsflüssigkeitschromatographie-Massenspektrometrie. In Proben aus Henndorf wurde kein Lolitrem B nachgewiesen. DW – Trockengewicht.

**Table 4.** Descriptive statistics for mycotoxins within grass samples (*Festuca*, *Lolium*, and Schedonorus species) determined by ultra-performance liquid chromatography–mass spectrometry. DW – dry weight; SD – standard deviation.

**Tabelle 4.** Deskriptive Statistik für Mykotoxine in Grasproben (*Festuca*-, *Lolium*- und *Schedonorus*-Arten), bestimmt mittels Ultra-Hochleistungsflüssigkeitschromatographie-Massenspektrometrie. DW – Trockengewicht; SD – Standardabweichung.

| Variable | Either | Ergovaline | Lolitrem B |
|---|---|---|---|
| Tested plots where present (%) | 35.8 | 26.0 | 19.1 |
| Positive samples (%) | 25.5 | 16.9 | 13.2 |
| Median content of positive samples (ng/g DW) | - | 136 | 45 |
| Maximum content of samples (ng/g DW) | - | 3395 | 1088 |
| SD of concentration in positive samples | - | 621 | 214 |
| Most common host | *Festuca rubra* aggr. | *Festuca rubra* aggr. | *Lolium perenne* |

At sample level, only the percent cover of grass species in the plot was predictive of mycotoxin occurrence in *Festuca rubra* aggr. samples for both mycotoxins combined (estimate 0.074, $p = 0.018$) or ergovaline presence specifically (estimate 0.073, $p = 0.05$; Table 5). For samples of *Lolium perenne*, higher nitrogen EIVEs in the vegetation were associated with lower chances of mycotoxin occurrence (estimate -1.14, $p = 0.034$). Higher percent covers of *Festuca*, *Lolium,* and *Schedonorus* species in the plots from which *Lolium perenne* samples were taken were negatively associated with ergovaline presence (estimate -0.016, $p = 0.003$). For individual samples, there was no significant relationship between the cover of the tested species within the plot and mycotoxin occurrence within the tested species.

**Table 5.** *p*-values from the generalized linear mixed models at plot ($n$ = 44) and sample level (*n Festuca rubra* aggr. = 108, *n Lolium perenne* = 141). Significant values are marked with asterisks and highlighted blue or red. Blue – positive association of mycotoxin occurrence with grazing or higher EIVEs or biodiversity metrics; red – negative association of mycotoxin occurrence with grazing or higher EIVEs or biodiversity metrics. EIVE – ecological indicator value. Either – either ergovaline or lolitrem B; Ergov. – ergovaline; Lol. B – lolitrem B. Significance levels: * – $p \leq 0.05$; ** – $p \leq 0.01$; *** – $p \leq 0.001$.

**Tabelle 5.** *p*-Werte aus den verallgemeinerten linearen gemischten Modellen auf Graslandebene ($n$ = 44) und Probenebene (*n Festuca rubra* aggr. = 108, *n Lolium perenne* = 141). Signifikante Werte sind mit Sternchen gekennzeichnet und blau oder rot hervorgehoben. Blau – positiver Zusammenhang zwischen dem Auftreten von Mykotoxinen und Beweidung oder höheren EIVEs oder Biodiversitätsmetriken; rot – negativer Zusammenhang zwischen dem Auftreten von Mykotoxinen und Beweidung oder höheren EIVEs oder Biodiversitätsmetriken. EIVE – ökologischer Indikatorwert. Either – entweder Ergovalin oder Lolitrem B; Ergov. – Ergovalin; Lol. B – Lolitrem B. Signifikanzniveaus: * – $p < 0{,}05$; ** – $p < 0{,}01$; *** – $p < 0{,}001$.

| | Plots | | *Festuca rubra* aggr. samples | | | *Lolium perenne* samples | | |
|---|---|---|---|---|---|---|---|---|
| Predictor variable | Either | Ergov. | Either | Ergov. | Lol. B | Either | Ergov. | Lol. B |
| Land use | 0.675 | 0.979 | 0.574 | 0.873 | 0.158 | 0.339 | 0.059 | 0.640 |
| Temperature EIVE | 0.169 | 0.165 | 0.203 | 0.118 | 0.885 | 0.591 | 0.913 | 0.674 |
| Soil moisture EIVE | 0.047* | 0.055 | 0.445 | 0.199 | 0.240 | 0.284 | 0.281 | 0.617 |
| Soil nitrogen EIVE | 0.326 | 0.434 | 0.562 | 0.495 | 0.324 | 0.034* | 0.363 | 0.101 |
| *Poaceae* cover (%) | 0.318 | 0.756 | 0.018* | 0.050* | 0.406 | 0.909 | 0.165 | 0.634 |
| *Lolium, Festuca,* and *Schedonorus* cover (%) | 0.935 | 0.441 | 0.216 | 0.137 | 0.191 | 0.425 | 0.003** | 0.987 |
| Species richness in 10 $m^2$ | 0.651 | 0.733 | 0.429 | 0.132 | 0.834 | 0.929 | 0.172 | 0.908 |
| Shannon index | 0.753 | 0.902 | 0.601 | 0.191 | 0.906 | 0.763 | 0.135 | 0.711 |
| Shannon evenness | 0.097 | 0.212 | 0.429 | 0.957 | 0.486 | 0.409 | 0.313 | 0.252 |

# 5. Discussion

## 5.1 Average biodiversity, with conservation potential according to land-use intensity

The surveyed grasslands and sites varied widely in species richness. The mean vascular plant species richness of 25.1 in 10 $m^2$ is comparable to the average for mesic, semidry, and wet grasslands in the countries of this study (26.9 species in 10 $m^2$, $n$ = 380; Grassplot Diversity Explorer v. 2.10, https://edgg.org/databases/GrasslandDiversityExplorer, EDGG 2025; see Biurrun et al. 2021). The maximum of 48 species in 10 $m^2$ in our study is exceptionally high. We generally found higher richness in semidry or wet grasslands with low-intensity use. The occurrence of national Red List species within the dataset and the observed qualitative patterns in species richness demonstrate the potential of low-intensity grazing with horses to promote grassland biodiversity (e.g. Köhler et al. 2016).

Varying soil nitrogen EIVEs are likely partly due to differences in management, with some grasslands being apparently used and fertilized more intensively than others. Unfortunately, land-use intensity could not be precisely characterized with the information available. Strong differences in land-use intensity which could not be quantified or included in the models, along with the variability of environmental conditions among and within sites, could be the reason why no significant effect of land-use type on the tested response

variables could be detected. Of the tested EIVEs, soil nitrogen content and soil moisture had the broadest explanatory power for species assemblages plotted in the DCA, underlining the importance of edaphic factors and differences in land-use intensity.

As expected, Poaceae species were dominant in the plots. Common endophyte hosts (*Festuca*, *Lolium*, and *Schedonorus* species) made up a little over 1/3 of the average Poaceae cover. This differs from most studies on endophytes, which were carried out in monocultures or grasslands where these species are strongly dominant. Animal intoxication is mostly known from species-poor grasslands (König et al. 2018). The percent cover of common endophyte hosts decreased with increasing species richness. We assert that species-rich grasslands may provide a protective effect against endophyte toxicosis by reducing the concentration of mycotoxins in the fodder (see also Malinowski & Belesky 2006). Intensive grassland management practices such as overseeding with grasses and excessive fertilization can lead to decreases in diversity and increases in the cover of grasses through the disappearance of establishment gaps for dicots, which may increase the risk of endophyte toxicosis in livestock.

### 5.2 Endophytes are broadly present in temperate Europe, but appear to present only limited risks for livestock in semi-natural grasslands

We were able to detect endophyte infection as indicated by mycotoxin occurrence in grasslands covering a broad range of environmental conditions. The studied mycotoxins were detected at all seven sites in four countries covering approximately 3° of latitude and 14° of longitude, in 50% of the tested grasslands and 35.8% of the tested plots. References have yielded varying results for endophyte prevalence in Europe. In an early study of 523 locations in 20 countries, Lewis et al. (1997) detected endophytes in 15 countries and 62% of the sites, with only 14 sites showing infection rates of 51–100%. Jensen & Roulund (2004) found endophytes at 77% of Danish locations within varying habitat types, with local infection rates from 4–82%. A review of Polish studies estimated infection in about 70% of grasslands in Poland (Żurek et al. 2012). König et al. (2018) found fungal endophytes in 66% of farms within three regions of Germany. Local infection rates were similar to those found within other German locations (Dobrindt et al. 2013, Oldenburg 1997), varying from 5.4% to 35.7% in tested samples (vs. 6% to 28%). Drawing on a large set of endophyte infection records, the models of Semmartin et al. (2015) estimate an approximate 40–50% endophyte infection at the latitudes included in our study. Thus, our results for mycotoxin prevalence show comparable rates at the local level to those found in the literature but suggest higher infection rates at larger scales than had generally been observed in previous studies, since not a single site was unaffected.

Endophyte mycotoxins have been shown to be responsible for disease in livestock, includeing ryegrass staggers and fescue toxicosis (e.g. Blythe et al. 2007, Schardl et al. 2012). In horses, the effects of endophyte toxicosis are particularly severe in pregnant mares and their foals (Putnam et al. 1991, Duringer et al. 2013). The threshold for ergovaline toxicity in horses (300–500 ng/g DW; Duringer et al. 2013) was exceeded in 17 cases, with occurrence in all four countries. These samples consisted mostly of *Festuca rubra* aggr. (9/17). Only in a few cases did species whose samples exceeded thresholds for toxicity make up over 25% of the vegetation cover. No samples exceeded the critical threshold for lolitrem B in cattle and sheep (1800–2000 ng/g DW; Duringer et al. 2013, see also Johnstone et al. 2012) (Fig. 4). To our knowledge, a threshold for lolitrem B toxicity in horses has yet to be determined.

Although incidences of endophyte toxicosis in European livestock appear to be rare as of yet (Repussard et al. 2014, König et al. 2018), the possible impact of these endophytes on European agriculture and animal husbandry requires further study – especially considering the trend of ongoing agricultural intensification. Endophytes are apparently long-lived in perennial grasses, with infected tussocks persisting for decades (Bacon & White 2000).

In interpreting the mycotoxin data, several limitations of our dataset must be considered. The presence of mycotoxins is only indirectly related to the occurrence of *Epichloe* endophytes, since ergovaline can also be produced by other fungal genera. It is also unclear whether lolitrem B is produced by other fungal genera. Due to time constraints, collected plant samples differed in phenology and/or tissue type. Our results also may be affected by individual variation in mycotoxin production (Ball et al. 1991) in cases where only small populations could be sampled, but samples consisting of only one or few individual plants were rare. Mycotoxin quantities are not strictly tied to endophyte quantity or vigor, and the plants of different age and condition, as well as different parts of the same individual plant, have been shown to vary in the concentration of individual mycotoxins (Spiering et al. 2005, Fuchs et al. 2013, Repussard et al. 2014). However, since our models test effects on simple occurrence rather than mycotoxin concentration itself, we assume that these limitations have not strongly affected our results. The fact that samples were collected during the warmer months when mycotoxin concentrations peak (Ball et al. 1991, Son et al. 2023) also helps minimize possible sources of error. Where possible, all collected tissue types were submitted for mycotoxin analysis.

### 5.3 *Festuca rubra* aggr. contains mycotoxins more often than *Lolium perenne* and *Schedonorus arundinaceus*

Most of the literature on grass-endophyte symbioses has focused on *Lolium perenne* and *Schedonorus arundinaceus*, species which are of global economic importance as fodder grasses and have been involved in high-profile cases of endophyte toxicosis in livestock. However, in our dataset, *Festuca rubra* aggr. was the species which most commonly contained mycotoxins, at rates exceeding twice that of *Lolium perenne* and *Schedonorus arundinaceus*. Additionally, our study marks the first time that lolitrem B has been detected in *Festuca rubra* aggr. to our knowledge. *Festuca rubra* aggr. is a globally important turf grass and ubiquitous species in grasslands throughout Europe, found in a wide range of habitats (Leinonen et al. 2019). Worldwide, endophyte infection has commonly been detected in this species (Saha et al. 1987, Clay & Schardl 2002), although infection rates vary among European populations and infection appears absent in some regions (Zabalgogeazcoa et al. 1999, Wäli et al. 2007, Dirihan et al. 2016). Should endophyte toxicosis in livestock emerge as a problem in temperate Europe, we recommend that due attention be paid to grass-endophyte symbioses in *Festuca rubra* aggr.

### 5.4 Little evidence of a relationship between ecological conditions and endophyte infection

Models analyzing mycotoxin occurrence at plot and sample level showed conflicting results. Within the 44 plots with complete testing of all *Festuca*, *Lolium,* and *Schedonorus* species, mycotoxin occurrence was associated only with higher soil moisture EIVEs. This is surprising given evidence connecting infection prevalence to drought conditions and low water availability (e.g. Lewis et al. 1997, Semmartin et al. 2015). Endophyte infection is

thought to confer drought tolerance to host grasses (Malinowski & Beleski 2006, Cheplick & Faeth 2009), giving them a fitness advantage. In recent years, possible mechanisms conferring such resistance have been demonstrated experimentally (e.g. Raeisi-Vanani et al. 2025). However, drought tolerance in host grasses has not always been convincingly demonstrated in field studies (Malinowski & Belesky 2006). Indeed, Ahlholm et al. (2002) found that infected *Festuca rubra* aggr. showed poorer growth than uninfected plants under low water availability. This suggests that the effects of water availability on grass-endophyte symbioses may not be so straightforward. Further studies on endophyte prevalence in grassland vegetation considering interactions between water availability and temperature may help reveal overall patterns.

Differences in mycotoxin prevalence between grazed and mown grasslands could not be found, either at plot or sample level. This again contradicts a widespread view on grass endophytes, which connects increased endophyte infection to intensive grazing (Gwinn et al. 1998, Bastias et al. 2017) based on the role of mycotoxins in deterring herbivory. Even in natural communities, grazing has been associated with higher endophyte infection frequencies (Jensen & Roulund 2004, Koh & Hik 2007, Dirihan et al. 2016). However, studies are sparse and contradictory; for example, Dobrindt et al. (2013) found no influence of land-use type or intensity on endophyte prevalence in Germany. Our results are particularly interesting in the implication that even very selective grazers such as horses do not necessarily lead to increases in endophyte dominance.

At sample level, results differed between host species and mycotoxin. For samples of *Lolium perenne*, mycotoxin occurrence was associated with low nitrogen availability. This is surprising given that the ostensible benefits of endophytes on host grasses appear more pronounced in nutrient-rich environments, with experimental evidence for increasing advantages of infection for hosts at higher nutrient levels (Arachevaleta et al. 1989, Cheplick et al. 1989, Ahlholm et al. 2002), confirmed by distribution patterns in wild populations (Semmartin et al. 2015). The fact that temperature EIVEs showed no predictive power for mycotoxin occurrence also conflicts with much of the literature on grass-endophyte symbioses, which link increased prevalence to warm, dry conditions (Malinowski & Belesky 2006). Endophyte infection appears to be greater in the warmer, dryer regions of Europe, with infections in temperate grasses correlating with mean annual temperature (Semmartin et al. 2015), although this finding is not universal (Leinonen et al. 2019).

For samples of *Festuca rubra* aggr., the percent cover of grass species present in the plot from which the sample was collected was related to both ergovaline presence alone and the presence of both mycotoxins together, with stronger grass dominance associated with mycotoxin occurrence. This is in line with the hypothesis that endophyte infection may increase competitive ability and host dominance in the vegetation, despite the fact that most studies have focused on host species other than *Festuca rubra* aggr. Lewis et al. (1997) found a slight but significant correlation between level of endophyte infection in *Lolium* spp. and the abundance of these taxa in the sward. However, ergovaline presence in *Lolium perenne* was rather associated with lower dominance of species of *Festuca*, *Lolium,* and *Schedonorus* species within the plot. These contradictory results may be due to species-specific effects on host ecology. In contrast to species dominance, biodiversity showed no significant effects on mycotoxin prevalence, either at plot level or sample level. In a succession experiment, Clay & Holah (1999) were able to associate endophyte infection with increased competitive ability and thus host dominance in the stand, resulting in lower species richness. No such relationship could be observed in our study. Indeed, Spyreas et al. (2001)

suggest on the basis of the results from their own succession experiment that a simple negative relationship between endophyte infection and plant diversity is unlikely to be universal.

In conclusion, we find no strong or consistent effects of abiotic conditions on mycotoxin prevalence, and no relationship between mycotoxin occurrence in the sward and community diversity. This is in line with evidence that the effects of infection on hosts are strongly dependent on host and endophyte genotype, and that local ecology can tip the symbiosis between mutualism and parasitism (Cheplick et al. 1989, Ahlholm et al. 2002). As such, abiotic factors do not always impose selective pressure on the grass-endophyte symbiosis and cannot be consistently linked to differences in host fitness with infection (Leinonen et al. 2019). Metapopulation models by Saikkonen et al. (2002) predict that grass-endophyte symbioses can persist in nature in the absence of fitness benefits for the host, even with incomplete transmission of infection to offspring. This would explain how these grass-endophyte symbioses have been observed over a broad range of environmental conditions throughout Europe, in our study and in many others over the past decades.

## Erweiterte deutsche Zusammenfassung

**Einleitung** – Halbnaturliches Grasland beherbergt eine hohe Artenvielfalt (Dengler et al. 2014), wird jedoch vielerorts in Europa durch Landnutzungsintensivierung oder-aufgabe bedroht (Török & Dengler 2018, Valkó et al. 2018). Ein aktuelles Forschungsgebiet, das sowohl für Graslandökosysteme als auch für die landwirtschaftliche Tierhaltung relevant ist, beschäftigt sich mit der Symbiose zwischen *Epichloe*-Pilzen (im Folgenden: «Endophyten») und Gräsern der Gattungen *Festuca*, *Lolium* und *Schedonorus*. Diese Endophyten produzieren für Weidetiere toxische Alkaloide und werden mit bestimmten Fitnessvorteilen für die Wirtsgräser in Verbindung gebracht, was möglicherweise zu einer Verringerung der Artenvielfalt führt (Clay & Holah 1999, Malinowski & Belesky 2006). Die Gras-Endophyt-Symbiose scheint sowohl von Herbivorie als auch von abiotischen Faktoren wie Temperatur und Wasserverfügbarkeit beeinflusst zu werden (Cheplick & Faeth 2009). Diese Symbiosen wurden allerdings bisher nur selten in (halb-)natürlichen Lebensräumen untersucht (Saikkonen et al. 2006, König et al. 2018, Leinonen et al. 2019). Mit dieser Studie wollten wir daher den Wissensstand über das System Endophyten-Grasland-Weidetiere im temperaten Europa verbessern, mit zwei Hauptzielen: (1) Die Verbreitung dieser Endophyten in für die Pferdehaltung genutzten Wiesen und Weiden zu quantifizieren, mittels Analyse des Mykotoxinvorkommens. (2) Die Umweltbedingungen und Biodiversitätsmuster in solchen Wiesen und Weiden zu charakterisieren, um mögliche Zusammenhängen mit dem Endophytenbefall zu erkennen.

**Untersuchungsgebiet** – Die Daten dieser Studie erhoben wir zwischen 250–650 m ü. M. in vier Ländern im temperaten Europa: Frankreich, Deutschland, Österreich und Ungarn (Abb. 1). Innerhalb dieser Länder sind die sieben Untersuchungsstandorte Teil eines Netzwerks von Tierheimen, in denen Pferde gehalten werden (Tab. 1). Gemäß der pflanzensoziologischen Typologie von Mucina et al. (2016) gehörte die große Mehrheit der Grasländer zur Klasse *Molinio-Arrhenatheretea*, meist zur Ordnung *Arrhenatheretalia elatioris* mit den Verbänden *Arrhentherion elatioris* und *Cynosurion cristati* (Abb. 2).

**Methoden** – In den Jahren 2021–2024 haben wir 310 Vegetationsaufnahmen von 10 m² Größe in den sieben Untersuchungsgebieen erhoben (Abb. 1, Tab. 1). Pro Untersuchungsgebiet haben wir fünf bis 11 Grasländer (etwa zur Hälfte Wiesen und Weiden) mit jeweils fünf Vegetationsaufnahmen beprobt. Das Samplingdesign pro Untersuchungsgebiet reichte von stratifiziert-systematisch bis subjektiv. Pro Vegetationsaufnahme haben wir alle Gefässpflanzen-Arten und ihre prozentuale Deckungswerte erfasst (zu den Vorteilen dieser Methode, siehe Dengler & Dembicz 2023).

Für die Gesamtvegetation jeder Vegetationsaufnahme haben wir drei Biodiversitätsmasse (Artenzahlen, Shannon-Indizes, Shannon-Evenness) berechnet. Auf der Grundlage des paneuropäischen Systems EIVE v. 1.0 (Dengler et al. 2023) haben wir auch quadratwurzelgewichtete ökologische Zeigerwerte (auf der Skala 0–10) berechnet (Lichtverfügbarkeit, Temperatur, Bodenreaktion, Bodenfeuchte und Bodenstickstoffgehalt). Ökologische und Diversitätsunterschiede zwischen Untersuchungsstandorten und Nutzungstypen haben wir mit Varianzanalyse (ANOVA) bzw. mit gemischten Modellen getestet. Um die allgemeinen Muster in den floristischen Daten zu visualisieren, haben wir eine Ordination der Vegetationsaufnahmen unter mittels trendbereinigter Korrespondenzanalyse (DCA) berechnet.

In einer Untergruppe von 204 Vegetationsaufnahmen haben wir Proben der Gras-Gattungen *Festuca*, *Lolium* und *Schedonorus* auf die Mykotoxine Ergovalin und Lolitrem B mittels Ultra-Hochleistungsflüssigkeitschromatographie-Massenspektrometrie untersucht. Diese Gattungen umfassen wichtige Futterpflanzen bzw. bekannte Wirte von *Epichloe* (Cheplick & Faeth 2009). Die zwei Mykotoxine haben wir aufgrund ihrer toxischen Wirkung auf Nutztiere und ihrer Korrelation mit dem Vorhandensein von Endophytenmyzel ausgewählt (Spiering et al. 2005). Die Mykotoxin-Analysen ergaben binomiale Daten, die das Mykotoxinenvorkommen anzeigen, und kontinuierliche Konzentrationsmessungen. Um mögliche ökologischen Einflüsse auf das Mykotoxinvorkommen zu untersuchen, haben wir verallgemeinerte gemischte Modelle auf Vegetationsaufnahme- und Probenebene berechnet.

**Ergebnisse** – Der mittlere Artenreichtum betrug 25,1 Arten in 10 $m^2$, mit einem Maximum von 48 Arten in 10 $m^2$. Es konnte kein signifikanter Zusammenhang zwischen Biodiversitätsmassen und Nutzungstyp festgestellt werden (Abb. 3, Tab. 2). Die meisten ökologischen Bedingungen variierten zwischen den Untersuchungsstandorten (Tab. 2), aber die Untersuchungsstandorte waren überwiegend nährstoffreich (Abb. 5d). Der Nutzungstyp hatte keinen signifikanten Einfluss auf die getesteten EIVEs (Tab. 2). DCA-Achsen 1 und 3 erklärten 27 % bzw. 24 % der Varianz der floristischen Daten. DCA1 korrelierte am stärksten mit Stickstoffgehalt-EIVE ($r$ = 0,995), während DCA3 am stärksten mit Bodenfeuchte-EIVE ($r$ = 0,959) und Bodenreaktion-EIVE ($r$ = -0,956) korrelierte (Abb. 6). Die verschiedenen Länder und Landnutzungstypen zeigten keine klare Trennung in der Ordination.

Mykotoxine traten in allen Untersuchungsgebieten auf, teilweise mit sehr hohen Konzentrationen in der Biomasse (Abb. 7). Wir fanden in 35,8 % der 204 untersuchten Vegetationsaufnahmen Mykotoxine (Tab. 4). *Festuca rubra* aggr. wurde mehr als doppelt so häufig auf Mykotoxine positiv getestet als *Lolium perenne* und *Schedonorus arundinaceus* (45,3 % der Proben vs. 24,1 % und 11,5 %; Tab. 3). Auf Probenebene war der Befall mit höheren Bodenfeuchte-EIVEs und niedrigeren Stickstoffgehalt-EIVEs verbunden (Tab. 5). Die Deckung von Gräsern im Allgemeinen und die Dominanz der Wirtsarten (*Festuca*-, *Lolium*- und *Schedonorus*-Arten) zeigten gegensätzliche Auswirkungen auf Mykotoxinvorkommen.

Bei den 44 Vegetationsaufnahmen, auf denen alle Zieltaxa vollständig getestet wurden, waren höhere Bodenfeuchte-EIVEs mit einer höheren Wahrscheinlichkeit für Mykotoxinvorkommen verbunden (Tab. 5). Es konnten keine signifikanten Auswirkungen auf das Auftreten von Ergovalin festgestellt werden. Es gab keinen Zusammenhang den Temperaturzeigerwerten, der Biodiversität oder dem Landnutzungstyp und dem Mykotoxinvorkommen (Tab. 5).

**Diskussion** – Der mittlere Pflanzenartenreichtum entsprach in etwa dem Durchschnitt entsprechender Grasländer in den untersuchten Ländern (26,9 Arten in 10 $m^2$, $n$ = 380; gemäss Grassplot Diversity Explorer v. 2.10, https://edgg.org/databases/GrasslandDiversityExplorer, EDGG 2025; vgl. Biurrun et al. 2021). Das Vorkommen von Arten der nationalen Roten Liste und die beobachteten qualitativen Muster im Artenreichtum zeigen das Potenzial einer extensiven Beweidung mit Pferden für die Biodiversitätsförderung (Köhler et al. 2016).

*Festuca*-, *Lolium*- und *Schedonorus*-Arten machten etwas mehr als ein Drittel der durchschnittlichen Gräserdeckung aus. Dies unterscheidet sich von den meisten Studien zu Endophyten, die in Kulturen durchgeführt wurden, in denen diese Arten stark dominierten. Tiervergiftungen sind vor allem aus artenarmen Grasländern bekannt (König et al. 2018). Die prozentuale Deckung von diesen häufigen Endophytenwirten nahm mit zunehmender Artenvielfalt ab. Das Artenreichtum könnte daher eine

schützende Wirkung gegen Endophytenvergiftungen bei Weidetieren haben, indem es die Mykotoxinkonzentration in der Biomasse möglicherweise verdünnt (vgl. Malinowski & Belesky 2006).

Mykotoxine traten unter einer Vielzahl von Umweltbedingungen auf. Die Literatur liefert unterschiedliche Angaben zur Prävalenz von Endophyten in Europa, aber die Befallsrate an unterschiedlichen Untersuchungsstandorte in Europa bewegt sich meist zwischen 60–80 % (z. B. Lewis et al. 1997, Żurek et al. 2012, König et al. 2018). Unsere Resultate deuten also auf eine höhere internationale Befallsrate hin, als bisher vermutet, da kein einziges Untersuchungsgebiet davon verschont blieb. Die lokalen Befallsraten innerhalb der Untersuchungsgebieten waren aber mit Werten aus der Literatur vergleichbar (z. B. Jensen & Roulund 2004, Dobrindt et al. 2013, Oldenburg 1997).

Der Schwellenwert für die Ergovalin-Toxizität für Pferde (300–500 ng/g DW; Duringer et al. 2013) wurde in 17 Fällen überschritten, verteilt auf alle vier Länder. Obwohl Fälle von Endophyten-Toxikose bei europäischen Weidetieren bislang selten zu sein scheinen (Repussard et al. 2014, König et al. 2018), sollten die möglichen Auswirkungen dieser Endophyten auf die europäische Landwirtschaft und Tierhaltung weiter untersucht werden. Der Großteil der Literatur über Gras-Endophyt-Symbiosen konzentriert sich auf *Lolium perenne* und *Schedonorus arundinaceus*, beide global wichtige Futtergräser. In unserem Datensatz war jedoch *Festuca rubra* aggr. das Taxon, das am häufigsten Mykotoxine enthielt. Darüber hinaus ist unsere Studie unseres Wissens die erste, in der Lolitrem B in *Festuca rubra* aggr. nachgewiesen wurde. Sollte die Endophytentoxikose bei Weidetieren in den gemäßigten Regionen Europas zu einem Problem werden, empfehlen wir, dem Endophytenbefall in *Festuca rubra* aggr. gebührende Aufmerksamkeit zu schenken.

Die einzelnen Modelle zum Mykotoxinvorkommen zeigten widersprüchliche Ergebnisse. In den 44 Vegetationsaufnahmen, in denen alle *Festuca*-, *Lolium*- und *Schedonorus*-Arten vollständig getestet wurden, konnten wir das Auftreten von Mykotoxinen nur mit höheren Bodenfeuchte-Zeigerwerten verbinden, trotz Hinweise aus der Literatur, dass eine höhere Befallsrate mit tiefer Wasserverfügbarkeit zusammenhängt (z. B. Lewis et al. 1997, Semmartin et al. 2015). Bei Proben von *Lolium perenne* war das Mykotoxinvorkommen mit einem geringen Stickstoff-Zeigerwert verbunden, im Gegensatz zu vielen anderen Studien (z. B. Arachevaleta et al. 1989, Cheplick et al. 1989, Ahlholm et al. 2002). Die Tatsache, dass die Temperatur-Zeigerwerte keine Vorhersagekraft fürs Mykotoxinvorkommen hatten, steht auch im Widerspruch zu einem Großteil der Literatur, die eine erhöhte Befallsrate mit warmen, trockenen Bedingungen in Verbindung bringt (z. B. Malinowski & Belesky 2006). In Gegensatz zu Clay & Holah (1999) konnten wir keinen Zusammenhang zwischen Endophytenbefall und erhöhter Konkurrenzstärke und damit Dominanz der Wirtsgräser im Bestand, noch verringerten Artenreichtum beobachten. Wir vermuten, dass ein negativer Zusammenhang zwischen Endophytenbefall und Pflanzenartenreichtum nicht universell ist (siehe auch Spyreas et al. 2001).

Zusammenfassend lässt sich sagen, dass wir keine starken oder konsistenten Auswirkungen abiotischer Bedingungen oder Biodiversität auf das Mykotoxinvorkommen feststellen konnten. Dies steht im Einklang mit der Erkenntnis, dass die Auswirkungen der Symbiose auf den Wirt stark vom Genotyp des Wirts und des Endophyten abhängen, und dass die lokale Ökologie die Symbiose stark beeinflusst (Cheplick et al. 1989, Ahlholm et al. 2002). Metapopulationsmodelle von Saikkonen et al. (2002) besagen, dass die Gras-Endophyt-Symbiose in der Natur auch ohne Fitnessvorteile für den Wirt bestehen bleiben kann. Dies würde erklären, warum diese Gras-Endophyt-Symbiose in unserer Studie und in vielen anderen Studien der letzten Jahrzehnte unter einer Vielzahl von Umweltbedingungen beobachtet wurde.

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## Author contributions

The concept of the initial regional projects, choice of study sites, and sampling design was defined by V.M. and R.V. The sampling was carried out by H.S., M.B., B.D., K.S., O.V., and J.D. with inputs from V.M. and R.V. H.S. carried out the analyses and drafted the manuscript under the guidance of J.D. All authors checked, improved, and approved the manuscript.

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## Supplements

**Additional supporting information may be found in the online version of this article.**

**Zusätzliche unterstützende Information ist in der Online-Version dieses Artikels zu finden.**

**Supplement E1.** Tested grass samples and their mycotoxin concentrations.
**Anhang E1.** Getestete Grasproben und ihre Mykotoxin-Werte.

**Supplement E2.** Lab protocol for mycotoxin detection.
**Anhang E2.** Laborprotokoll zum Mykotoxin-Nachweis.

**Supplement E3.** Nationally red-listed species found in the plots.
**Anhang E3.** Vorkommende Arten der nationalen Roten Listen.

**Supplement E4.** Rates of mycotoxin occurrence per site.
**Anhang E4.** Mykotoxin-Positivitätsraten pro Gebiet (Betrieb).

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**Supplement E1.** Tested grass samples and their mycotoxin concentrations. Phenological stages according to Meier (2001).

**Anhang E1.** Getestete Grasproben und ihre Mykotoxin-Werte. Phänologische Stadien nach Meier (2001).

| Sample ID | Plot | Country | Site | Grass species | Phenology | Lolitrem B (ng/g DW) | Ergovaline (ng/g DW) |
|---|---|---|---|---|---|---|---|
| 299 | EN_FR_1.1 | FR | Trévol | *Festuca rubra* aggr. | 29 | 0 | 0 |
| 300 | EN_FR_1.1 | FR | Trévol | *Lolium perenne* | 29 | 11,89 | 0 |
| 301 | EN_FR_1.2 | FR | Trévol | *Festuca rubra* aggr. | 29 | 0 | 0 |
| 302 | EN_FR_1.4 | FR | Trévol | *Festuca rubra* aggr. | 29 | 0 | 0 |
| 303 | EN_FR_1.5 | FR | Trévol | *Festuca rubra* aggr. | 29 + 99 | 31,27 | 0 |
| 304 | EN_FR_1.5 | FR | Trévol | *Lolium perenne* | 69 | 74,37 | 0 |
| 305 | EN_FR_2.2 | FR | Trévol | *Festuca rubra* aggr. | 29 + 99 | 0 | 0 |
| 306 | EN_FR_2.3 | FR | Trévol | *Lolium perenne* | 29 | 0 | 0 |
| 307 | EN_FR_2.4 | FR | Trévol | *Lolium perenne* | 69 | 0 | 0 |
| 308 | EN_FR_3.1 | FR | Trévol | *Festuca rubra* aggr. | 29 + 99 | 0 | 0 |
| 309 | EN_FR_3.3 | FR | Trévol | *Lolium perenne* | 29 + 99 | 0 | 0 |
| 310 | EN_FR_4.1 | FR | Trévol | *Festuca rubra* aggr. | 93 | 0 | 0 |
| 311 | EN_FR_4.1 | FR | Trévol | *Lolium perenne* | 29 + 99 | 0 | 0 |
| 312 | EN_FR_4.2 | FR | Trévol | *Lolium perenne* | 29 | 126,04 | 0 |
| 314 | EN_FR_4.3 | FR | Trévol | *Lolium perenne* | 29 + 99 | 1088,08 | 0 |
| 315 | EN_FR_4.4 | FR | Trévol | *Festuca rubra* aggr. | 29 | 663,46 | 0 |
| 316 | EN_FR_5.2 | FR | Trévol | *Festuca rubra* aggr. | 7 | 0 | 0 |
| 317 | EN_FR_5.3 | FR | Trévol | *Lolium perenne* | 65–69 | 0 | 0 |
| 318 | EN_FR_5.5 | FR | Trévol | *Festuca rubra* aggr. | 29 + 99 | 0 | 0 |
| 192 | C_ct1 | FR | Trévol | *Lolium perenne* | 58 | 0 | 0 |
| 193 | C_ct1 | FR | Trévol | *Schedonorus arundinaceus* | 58 | 0 | 0 |
| 194 | C_ct1 | FR | Trévol | *Festuca rubra* aggr. | 59 | 0 | 0 |
| 195 | C_ct2 | FR | Trévol | *Lolium perenne* | NA | 0 | 516,59 |
| 196 | C_ct2 | FR | Trévol | *Festuca rubra* aggr. | 59 | 0 | 0 |
| 197 | C_ct2 | FR | Trévol | *Schedonorus arundinaceus* | 56 | 0 | 0 |
| 198 | C_ct3 | FR | Trévol | *Lolium perenne* | NA | 0 | 0 |
| 199 | C_ct3 | FR | Trévol | *Schedonorus arundinaceus* | 56 | 0 | 0 |
| 200 | C_ct4 | FR | Trévol | *Lolium perenne* | 58 | 0 | 0 |
| 201 | C_ct4 | FR | Trévol | *Schedonorus arundinaceus* | 54 | 0 | 0 |
| 202 | C_ct4 | FR | Trévol | *Festuca rubra* aggr. | 59 | 167,94 | 0 |
| 203 | C_ct5 | FR | Trévol | *Festuca rubra* aggr. | 59 | 0 | 0 |
| 204 | C_ct5 | FR | Trévol | *Schedonorus arundinaceus* | 56 | 0 | 0 |
| 205 | C_gh1 | FR | Trévol | *Lolium perenne* | 58 | 0 | 0 |
| 206 | C_gh1 | FR | Trévol | *Schedonorus arundinaceus* | 56 | 0 | 0 |
| 207 | C_gh2 | FR | Trévol | *Lolium perenne* | 58 | 0 | 0 |
| 208 | C_gh2 | FR | Trévol | *Festuca rubra* aggr. | 59 | 0 | 36,17 |
| 209 | C_gh2 | FR | Trévol | *Schedonorus arundinaceus* | 54 | 0 | 0 |
| 210 | C_gh3 | FR | Trévol | *Festulolium ×loliaceum* | 59 | 0 | 0 |
| 211 | C_gh3 | FR | Trévol | *Festuca rubra* aggr. | 59 | 0 | 0 |
| 212 | C_gh3 | FR | Trévol | *Schedonorus arundinaceus* | 56 | 0 | 0 |
| 213 | C_gh3 | FR | Trévol | *Lolium perenne* | 61 | 0 | 0 |
| 214 | C_gh4 | FR | Trévol | *Festuca rubra* aggr. | 65 | 2,46 | 44,88 |
| 215 | C_gh4 | FR | Trévol | *Lolium perenne* | 61 | 46,29 | 868,02 |
| 216 | C_gh4 | FR | Trévol | *Schedonorus arundinaceus* | 61 | 0 | 0 |
| 217 | C_gh5 | FR | Trévol | *Festuca rubra* aggr. | 69 | 27,54 | 0 |
| 218 | C_gh5 | FR | Trévol | *Schedonorus arundinaceus* | 61 | 0 | 0 |
| 219 | C_gh5 | FR | Trévol | *Lolium perenne* | 61 | 0 | 0 |
| 220 | C_rs1 | FR | Trévol | *Lolium perenne* | 61 | 29,35 | 0 |
| 221 | C_rs1 | FR | Trévol | *Festuca rubra* aggr. | 61 | 38,09 | 0 |
| 222 | C_rs2 | FR | Trévol | *Lolium perenne* | 52 | 0 | 0 |
| 223 | C_rs2 | FR | Trévol | *Schedonorus arundinaceus* | 61 | 0 | 0 |
| 224 | C_rs3 | FR | Trévol | *Festuca rubra* aggr. | 65 | 49,03 | 87,86 |
| 225 | C_rs3 | FR | Trévol | *Lolium perenne* | 61 | 4,4 | 0 |
| 226 | C_rs3 | FR | Trévol | *Schedonorus arundinaceus* | 65 | 0 | 0 |
| 227 | C_rs4 | FR | Trévol | *Lolium perenne* | NA | 0 | 0 |
| 228 | C_rs4 | FR | Trévol | *Schedonorus arundinaceus* | 59 | 0 | 0 |
| 229 | C_rs5 | FR | Trévol | *Schedonorus pratensis* | 59 | 0 | 0 |
| 230 | C_rs5 | FR | Trévol | *Schedonorus arundinaceus* | 59 | 0 | 0 |
| 231 | C_rs5 | FR | Trévol | *Lolium perenne* | NA | 27,18 | 93,98 |
| 232 | C_rs5 | FR | Trévol | *Festuca rubra* aggr. | NA | 0 | 0 |
| 233 | C_ss1 | FR | Trévol | *Schedonorus pratensis* | 56 | 0 | 0 |
| 234 | C_ss1 | FR | Trévol | *Festuca rubra* aggr. | 59 | 33,35 | 4,22 |

| Sample ID | Plot | Country | Site | Grass species | Phenology | Lolitrem B (ng/g DW) | Ergovaline (ng/g DW) |
|---|---|---|---|---|---|---|---|
| 235 | C_ss1 | FR | Trévol | *Lolium perenne* | 58 | 49,38 | 0 |
| 236 | C_ss1 | FR | Trévol | *Schedonorus arundinaceus* | 56 | 0 | 0 |
| 237 | C_ss3 | FR | Trévol | *Lolium perenne* | NA | 45,13 | 0 |
| 238 | C_ss3 | FR | Trévol | *Schedonorus arundinaceus* | NA | 0 | 0 |
| 239 | C_ss4 | FR | Trévol | *Festuca rubra* aggr. | 59 | 0 | 0 |
| 240 | C_ss4 | FR | Trévol | *Lolium perenne* | 58 | 179,43 | 32,34 |
| 241 | C_ss4 | FR | Trévol | *Schedonorus arundinaceus* | 59 | 0 | 0 |
| 242 | C_ss5 | FR | Trévol | *Lolium perenne* | 58 | 0 | 641,79 |
| 243 | C_ss5 | FR | Trévol | *Schedonorus arundinaceus* | 56 | 0,46 | 0 |
| 244 | E_ct1 | FR | Trévol | *Lolium perenne* | 58 + 59 | 10,91 | 0 |
| 245 | E_ct1 | FR | Trévol | *Schedonorus arundinaceus* | 59 | 0 | 0 |
| 246 | E_ct1 | FR | Trévol | *Festuca rubra* aggr. | 59 | 187,26 | 0 |
| 247 | E_ct2 | FR | Trévol | *Festuca rubra* aggr. | 59 | 9,85 | 34,71 |
| 248 | E_ct2 | FR | Trévol | *Schedonorus arundinaceus* | 59 | 0 | 0 |
| 249 | E_ct2 | FR | Trévol | *Lolium perenne* | 58 | 113,49 | 0 |
| 250 | E_ct3 | FR | Trévol | *Lolium perenne* | 58 | 0 | 0 |
| 251 | E_ct3 | FR | Trévol | *Festuca rubra* aggr. | 59 | 34,02 | 0 |
| 252 | E_ct3 | FR | Trévol | *Schedonorus arundinaceus* | 59 | 0 | 0 |
| 253 | E_ct4 | FR | Trévol | *Lolium perenne* | 58 | 0 | 0 |
| 254 | E_ct4 | FR | Trévol | *Festuca rubra* aggr. | 59 | 0 | 0 |
| 255 | E_ct4 | FR | Trévol | *Schedonorus arundinaceus* | 59 | 0 | 0 |
| 256 | E_ct5 | FR | Trévol | *Festuca rubra* aggr. | 59 | 7,26 | 40,16 |
| 257 | E_ct5 | FR | Trévol | *Schedonorus arundinaceus* | 59 | 0 | 0 |
| 258 | E_ct5 | FR | Trévol | *Lolium perenne* | 59 | 7,12 | 0 |
| 259 | E_gh1 | FR | Trévol | *Festuca rubra* aggr. | 59 | 0 | 0 |
| 260 | E_gh1 | FR | Trévol | *Lolium perenne* | 58 | 0 | 0 |
| 261 | E_gh1 | FR | Trévol | *Schedonorus arundinaceus* | 59 | 0 | 0 |
| 262 | E_gh2 | FR | Trévol | *Festuca rubra* aggr. | 59 | 0 | 12,05 |
| 263 | E_gh2 | FR | Trévol | *Schedonorus arundinaceus* | 59 | 0 | 0 |
| 264 | E_gh2 | FR | Trévol | *Lolium perenne* | 58 | 40,4 | 0 |
| 265 | E_gh3 | FR | Trévol | *Schedonorus arundinaceus* | 59 | 0 | 0 |
| 266 | E_gh3 | FR | Trévol | *Lolium perenne* | 58 | 8,78 | 0 |
| 267 | E_gh4 | FR | Trévol | *Schedonorus arundinaceus* | 59 | 0 | 0 |
| 268 | E_gh4 | FR | Trévol | *Lolium perenne* | 58 | 0 | 0 |
| 269 | E_gh4 | FR | Trévol | *Festuca rubra* aggr. | 59 | 0 | 0 |
| 270 | E_gh5 | FR | Trévol | *Lolium perenne* | 58 | 2,93 | 5,31 |
| 271 | E_gh5 | FR | Trévol | *Schedonorus arundinaceus* | 59 | 0 | 0 |
| 272 | E_rs1 | FR | Trévol | *Schedonorus arundinaceus* | 59 | 0 | 0 |
| 273 | E_rs1 | FR | Trévol | *Lolium perenne* | 59 | 0 | 137,88 |
| 274 | E_rs1 | FR | Trévol | *Festuca rubra* aggr. | 59 | 0 | 0 |
| 275 | E_rs2 | FR | Trévol | *Schedonorus arundinaceus* | 59 | 0 | 0 |
| 276 | E_rs2 | FR | Trévol | *Lolium perenne* | 58 | 24,44 | 0 |
| 277 | E_rs2 | FR | Trévol | *Festuca rubra* aggr. | 59 | 39,81 | 138,94 |
| 278 | E_rs3 | FR | Trévol | *Schedonorus arundinaceus* | 59 | 0 | 0 |
| 279 | E_rs3 | FR | Trévol | *Festuca rubra* aggr. | 59 | 77,14 | 0 |
| 280 | E_rs3 | FR | Trévol | *Lolium perenne* | 59 | 0 | 0 |
| 281 | E_rs3 | FR | Trévol | *Festulolium ×loliaceum* | 57 | 0 | 0 |
| 282 | E_rs4 | FR | Trévol | *Schedonorus arundinaceus* | 61 | 0 | 0 |
| 283 | E_rs4 | FR | Trévol | *Festuca rubra* aggr. | 59 | 10,65 | 0 |
| 284 | E_rs4 | FR | Trévol | *Lolium perenne* | 57 | 0 | 0 |
| 285 | E_rs5 | FR | Trévol | *Festuca rubra* aggr. | 59 | 0 | 0 |
| 286 | E_rs5 | FR | Trévol | *Schedonorus arundinaceus* | 61 | 0 | 0 |
| 287 | E_rs5 | FR | Trévol | *Lolium perenne* | 59 | 0 | 0 |
| 288 | E_ss1 | FR | Trévol | *Lolium perenne* | 59 | 0 | 0 |
| 289 | E_ss1 | FR | Trévol | *Schedonorus arundinaceus* | NA | 0 | 0 |
| 290 | E_ss2 | FR | Trévol | *Schedonorus arundinaceus* | 61 | 0 | 0 |
| 291 | E_ss2 | FR | Trévol | *Festuca rubra* aggr. | 59 | 0 | 0 |
| 292 | E_ss3 | FR | Trévol | *Schedonorus arundinaceus* | 59 | 0 | 0 |
| 293 | E_ss3 | FR | Trévol | *Festuca rubra* aggr. | 59 | 0 | 0 |
| 294 | E_ss4 | FR | Trévol | *Schedonorus arundinaceus* | 59 | 0 | 0 |
| 295 | E_ss4 | FR | Trévol | *Festuca rubra* aggr. | 59 | 0 | 0 |
| 296 | E_ss5 | FR | Trévol | *Lolium perenne* | 58 | 63,72 | 0 |
| 297 | E_ss5 | FR | Trévol | *Festuca rubra* aggr. | 59 | 0 | 0 |
| 298 | E_ss5 | FR | Trévol | *Schedonorus arundinaceus* | 59 | 0 | 0 |
| 1 | EN_BB_1.1 | DE | Braunenberg | *Lolium perenne* | 69 | 0 | 0 |
| 2 | EN_BB_1.1 | DE | Braunenberg | *Schedonorus arundinaceus* | 73 | 0 | 0 |
| 3 | EN_BB_1.1 | DE | Braunenberg | *Festuca rubra* aggr. | 73 | 0 | 311,93 |
| 4 | EN_BB_1.2 | DE | Braunenberg | *Lolium perenne* | 69 | 115,25 | 257,85 |
| 5 | EN_BB_1.2 | DE | Braunenberg | *Schedonorus arundinaceus* | 73 | 0 | 136,22 |

| Sample ID | Plot | Country | Site | Grass species | Phenology | Lolitrem B (ng/g DW) | Ergovaline (ng/g DW) |
|---|---|---|---|---|---|---|---|
| 6 | EN_BB_1.2 | DE | Braunenberg | *Festuca rubra* aggr. | 73 | 0 | 166,67 |
| 7 | EN_BB_1.3 | DE | Braunenberg | *Schedonorus arundinaceus* | 83 | 0 | 150 |
| 8 | EN_BB_1.3 | DE | Braunenberg | *Lolium perenne* | 71 | 0 | 0 |
| 9 | EN_BB_1.3 | DE | Braunenberg | *Festuca rubra* aggr. | 75 | 0 | 8,95 |
| 10 | EN_BB_1.4 | DE | Braunenberg | *Festuca rubra* aggr. | 75 | 0 | 87,98 |
| 11 | EN_BB_1.4 | DE | Braunenberg | *Schedonorus arundinaceus* | 77 | 0 | 2076,14 |
| 12 | EN_BB_1.4 | DE | Braunenberg | *Lolium perenne* | 71 | 96,35 | 0 |
| 13 | EN_BB_1.5 | DE | Braunenberg | *Lolium perenne* | 69 | 0 | 0 |
| 14 | EN_BB_1.5 | DE | Braunenberg | *Schedonorus arundinaceus* | 75 | 0 | 353,7 |
| 15 | EN_BB_2.1 | DE | Braunenberg | *Schedonorus pratensis* | 71 | 0 | 0 |
| 16 | EN_BB_2.1 | DE | Braunenberg | *Festuca rubra* aggr. | 13 | 0 | 0 |
| 18 | EN_BB_2.1 | DE | Braunenberg | *Lolium perenne* | 61 | 242,73 | 0 |
| 19 | EN_BB_2.1 | DE | Braunenberg | *Schedonorus arundinaceus* | 71 | 0 | 0 |
| 20 | EN_BB_2.2 | DE | Braunenberg | *Schedonorus arundinaceus* | 65 | 0 | 0 |
| 21 | EN_BB_2.2 | DE | Braunenberg | *Festuca rubra* aggr. | 13 | 0 | 0 |
| 22 | EN_BB_2.2 | DE | Braunenberg | *Schedonorus pratensis* | 65 | 0 | 0 |
| 23 | EN_BB_2.2 | DE | Braunenberg | *Lolium perenne* | 69 | 0 | 0 |
| 24 | EN_BB_2.3 | DE | Braunenberg | *Festuca rubra* aggr. | 13 | 0 | 0 |
| 25 | EN_BB_2.3 | DE | Braunenberg | *Schedonorus pratensis* | 65 | 0 | 0 |
| 26 | EN_BB_2.3 | DE | Braunenberg | *Schedonorus arundinaceus* | 65 | 0 | 0 |
| 27 | EN_BB_2.3 | DE | Braunenberg | *Lolium perenne* | 69 | 0 | 0 |
| 28 | EN_BB_2.4 | DE | Braunenberg | *Schedonorus arundinaceus* | 69 | 0 | 0 |
| 29 | EN_BB_2.4 | DE | Braunenberg | *Festuca rubra* aggr. | 13 | 0 | 0 |
| 30 | EN_BB_2.4 | DE | Braunenberg | *Lolium perenne* | 61 | 0 | 0 |
| 31 | EN_BB_2.5 | DE | Braunenberg | *Schedonorus pratensis* | 69 | 0 | 0 |
| 32 | EN_BB_2.5 | DE | Braunenberg | *Schedonorus arundinaceus* | 69 | 0 | 0 |
| 33 | EN_BB_2.5 | DE | Braunenberg | *Festuca rubra* aggr. | 13 | 0 | 0 |
| 34 | EN_BB_2.5 | DE | Braunenberg | *Lolium perenne* | 61 | 0 | 0 |
| 35 | EN_BB_3.1 | DE | Braunenberg | *Lolium perenne* | 83 | 0 | 0 |
| 36 | EN_BB_3.1 | DE | Braunenberg | *Schedonorus arundinaceus* | 87 | 0 | 0 |
| 37 | EN_BB_3.2 | DE | Braunenberg | *Lolium perenne* | 83 | 0 | 0 |
| 38 | EN_BB_3.2 | DE | Braunenberg | *Schedonorus arundinaceus* | 87 | 0 | 0 |
| 39 | EN_BB_3.3 | DE | Braunenberg | *Schedonorus arundinaceus* | 87 | 0 | 0 |
| 40 | EN_BB_3.3 | DE | Braunenberg | *Lolium perenne* | 83 | 0 | 0 |
| 41 | EN_BB_3.4 | DE | Braunenberg | *Lolium perenne* | 89 | 0 | 0 |
| 42 | EN_BB_3.4 | DE | Braunenberg | *Schedonorus arundinaceus* | 92 | 0 | 0 |
| 43 | EN_BB_3.5 | DE | Braunenberg | *Lolium perenne* | 75 | 0 | 3395,11 |
| 44 | EN_BB_3.5 | DE | Braunenberg | *Schedonorus arundinaceus* | 92 | 0 | 0 |
| 45 | EN_BB_4.1 | DE | Braunenberg | *Schedonorus arundinaceus* | 89 | 0 | 0 |
| 46 | EN_BB_4.1 | DE | Braunenberg | *Festuca rubra* aggr. | 13 | 0 | 181,43 |
| 47 | EN_BB_4.2 | DE | Braunenberg | *Lolium perenne* | 89 | 0 | 0 |
| 48 | EN_BB_4.2 | DE | Braunenberg | *Festuca rubra* aggr. | 13 | 0 | 144,98 |
| 49 | EN_BB_4.2 | DE | Braunenberg | *Schedonorus arundinaceus* | 92 | 0 | 898,55 |
| 50 | EN_BB_4.3 | DE | Braunenberg | *Schedonorus arundinaceus* | 89 | 0 | 124,19 |
| 51 | EN_BB_4.3 | DE | Braunenberg | *Festuca rubra* aggr. | 13 | 0 | 8,27 |
| 52 | EN_BB_4.4 | DE | Braunenberg | *Festuca rubra* aggr. | 13 | 0 | 189,09 |
| 53 | EN_BB_4.5 | DE | Braunenberg | *Festuca rubra* aggr. | 13 | 0 | 133,51 |
| 54 | EN_BB_5.1 | DE | Braunenberg | *Lolium multiflorum* | 73 | 0 | 0 |
| 55 | EN_BB_5.2 | DE | Braunenberg | *Lolium multiflorum* | 73 | 0 | 0 |
| 56 | EN_BB_5.3 | DE | Braunenberg | *Lolium multiflorum* | 73 | 0 | 0 |
| 57 | EN_BB_5.4 | DE | Braunenberg | *Lolium multiflorum* | 73 | 0 | 0 |
| 58 | EN_BB_5.4 | DE | Braunenberg | *Schedonorus arundinaceus* | 85 | 0 | 0 |
| 59 | EN_BB_5.5 | DE | Braunenberg | *Lolium multiflorum* | 73 | 0 | 0 |
| 60 | EN_BB_5.5 | DE | Braunenberg | *Schedonorus arundinaceus* | 85 | 0 | 0 |
| 103 | EN_HB_1.1 | DE | Hermersberg | *Schedonorus pratensis* | 69 | 0 | 0 |
| 104 | EN_HB_1.2 | DE | Hermersberg | *Schedonorus arundinaceus* | 13 | 0 | 0 |
| 105 | EN_HB_1.2 | DE | Hermersberg | *Schedonorus pratensis* | 12 | 0 | 0 |
| 106 | EN_HB_1.3 | DE | Hermersberg | *Schedonorus arundinaceus* | 13 | 0 | 0 |
| 107 | EN_HB_1.3 | DE | Hermersberg | *Schedonorus pratensis* | 13 | 0 | 0 |
| 108 | EN_HB_1.4 | DE | Hermersberg | *Schedonorus pratensis* | 13 | 0 | 0 |
| 109 | EN_HB_1.4 | DE | Hermersberg | *Schedonorus arundinaceus* | 13 | 0 | 0 |
| 110 | EN_HB_1.5 | DE | Hermersberg | *Schedonorus pratensis* | 13 | 0 | 0 |
| 111 | EN_HB_2.1 | DE | Hermersberg | *Lolium perenne* | 69 | 0 | 0 |
| 112 | EN_HB_2.2 | DE | Hermersberg | *Festuca rubra* aggr. | 12 | 0 | 0 |
| 113 | EN_HB_2.2 | DE | Hermersberg | *Schedonorus pratensis* | 13 | 0 | 0 |
| 114 | EN_HB_2.2 | DE | Hermersberg | *Lolium perenne* | 69 | 0 | 0 |
| 115 | EN_HB_2.3 | DE | Hermersberg | *Lolium perenne* | 69 | 0 | 0 |
| 116 | EN_HB_2.4 | DE | Hermersberg | *Lolium perenne* | 69 | 0 | 0 |
| 117 | EN_HB_2.4 | DE | Hermersberg | *Festuca rubra* aggr. | 13 | 0 | 0 |

| Sample ID | Plot | Country | Site | Grass species | Phenology | Lolitrem B (ng/g DW) | Ergovaline (ng/g DW) |
|---|---|---|---|---|---|---|---|
| 118 | EN_HB_2.5 | DE | Hermersberg | *Lolium perenne* | 69 | 217,04 | 0 |
| 119 | EN_HB_2.5 | DE | Hermersberg | *Schedonorus pratensis* | 13 | 0 | 0 |
| 120 | EN_HB_3.1 | DE | Hermersberg | *Schedonorus pratensis* | 13 | 0 | 0 |
| 121 | EN_HB_3.1 | DE | Hermersberg | *Festuca rubra* aggr. | 12 | 73,58 | 288,21 |
| 122 | EN_HB_3.2 | DE | Hermersberg | *Lolium perenne* | 93 | 0 | 0 |
| 123 | EN_HB_3.2 | DE | Hermersberg | *Schedonorus pratensis* | 93 | 0 | 0 |
| 124 | EN_HB_3.3 | DE | Hermersberg | *Schedonorus pratensis* | 13 | 0 | 0 |
| 125 | EN_HB_3.3 | DE | Hermersberg | *Lolium perenne* | 13 | 0 | 0 |
| 126 | EN_HB_3.4 | DE | Hermersberg | *Schedonorus pratensis* | 13 | 0 | 0 |
| 127 | EN_HB_3.4 | DE | Hermersberg | *Lolium perenne* | 13 | 0 | 0 |
| 128 | EN_HB_3.4 | DE | Hermersberg | *Festuca rubra* aggr. | 13 | 0 | 240 |
| 129 | EN_HB_3.5 | DE | Hermersberg | *Festuca rubra* aggr. | 13 | 0 | 265,83 |
| 130 | EN_HB_3.5 | DE | Hermersberg | *Schedonorus pratensis* | 13 | 0 | 0 |
| 131 | EN_HB_4.1 | DE | Hermersberg | *Festuca rubra* aggr. | 12 | 53,36 | 91,93 |
| 132 | EN_HB_4.1 | DE | Hermersberg | *Lolium perenne* | 69 | 0 | 0 |
| 133 | EN_HB_4.1 | DE | Hermersberg | *Schedonorus pratensis* | 13 | 6,58 | 5,24 |
| 134 | EN_HB_4.2 | DE | Hermersberg | *Festuca rubra* aggr. | 12 | 0 | 50,75 |
| 135 | EN_HB_4.2 | DE | Hermersberg | *Schedonorus pratensis* | 13 | 0 | 0 |
| 136 | EN_HB_4.2 | DE | Hermersberg | *Lolium perenne* | 69 | 0 | 0 |
| 137 | EN_HB_4.3 | DE | Hermersberg | *Lolium perenne* | 89 | 7,39 | 0 |
| 138 | EN_HB_4.3 | DE | Hermersberg | *Lolium perenne* | 69 | 0 | 0 |
| 139 | EN_HB_4.3 | DE | Hermersberg | *Lolium perenne* | 69 | 0 | 0 |
| 140 | EN_HB_4.3 | DE | Hermersberg | *Festuca rubra* aggr. | 12 | 0 | 233,16 |
| 141 | EN_HB_4.3 | DE | Hermersberg | *Schedonorus pratensis* | 13 | 0 | 0 |
| 142 | EN_HB_4.4 | DE | Hermersberg | *Festuca rubra* aggr. | 13 | 0 | 415,63 |
| 143 | EN_HB_4.4 | DE | Hermersberg | *Schedonorus pratensis* | 13 | 0 | 0 |
| 144 | EN_HB_4.5 | DE | Hermersberg | *Lolium perenne* | 69 | 0 | 0 |
| 145 | EN_HB_4.5 | DE | Hermersberg | *Schedonorus pratensis* | 13 | 0 | 0 |
| 146 | EN_HB_4.5 | DE | Hermersberg | *Festuca rubra* aggr. | 12 | 0 | 310,2 |
| 147 | EN_HB_5.1 | DE | Hermersberg | *Festuca rubra* aggr. | 12 | 0 | 11,83 |
| 148 | EN_HB_5.1 | DE | Hermersberg | *Schedonorus pratensis* | 13 | 0 | 0 |
| 149 | EN_HB_5.2 | DE | Hermersberg | *Schedonorus pratensis* | 13 | 0 | 0 |
| 150 | EN_HB_5.3 | DE | Hermersberg | *Schedonorus pratensis* | 13 | 0 | 0 |
| 151 | EN_HB_5.3 | DE | Hermersberg | *Festuca rubra* aggr. | 13 | 138,34 | 337,31 |
| 152 | EN_HB_5.4 | DE | Hermersberg | *Schedonorus pratensis* | 13 | 0 | 0 |
| 153 | EN_HB_5.5 | DE | Hermersberg | *Festuca rubra* aggr. | 13 | 0 | 48,05 |
| 154 | EN_HB_5.5 | DE | Hermersberg | *Schedonorus pratensis* | 13 | 0 | 0 |
| 155 | EN_KF_1.1 | DE | Kesselfeld | *Lolium perenne* | 77 | 0 | 0 |
| 156 | EN_KF_1.2 | DE | Kesselfeld | *Lolium perenne* | 87 | 0 | 0 |
| 157 | EN_KF_1.3 | DE | Kesselfeld | *Lolium perenne* | 87 | 0 | 0 |
| 158 | EN_KF_1.4 | DE | Kesselfeld | *Lolium perenne* | 87 | 0 | 0 |
| 159 | EN_KF_1.5 | DE | Kesselfeld | *Lolium perenne* | 73 | 0 | 0 |
| 160 | EN_KF_2.1 | DE | Kesselfeld | *Lolium perenne* | 85 | 0 | 0 |
| 161 | EN_KF_2.2 | DE | Kesselfeld | *Lolium perenne* | 77 | 0 | 0 |
| 162 | EN_KF_2.2 | DE | Kesselfeld | *Festuca rubra* aggr. | 75 | 0 | 0 |
| 163 | EN_KF_2.3 | DE | Kesselfeld | *Lolium perenne* | 87 | 0 | 0 |
| 164 | EN_KF_2.4 | DE | Kesselfeld | *Lolium perenne* | 73 | 0 | 0 |
| 165 | EN_KF_2.5 | DE | Kesselfeld | *Lolium perenne* | 85 | 0 | 0 |
| 166 | EN_KF_3.1 | DE | Kesselfeld | *Lolium perenne* | 85 | 0 | 0 |
| 167 | EN_KF_3.2 | DE | Kesselfeld | *Lolium perenne* | 85 | 0 | 9,04 |
| 168 | EN_KF_3.2 | DE | Kesselfeld | *Festulolium ×loliaceum* | 73 | 0 | 0 |
| 169 | EN_KF_3.3 | DE | Kesselfeld | *Festulolium ×loliaceum* | 71 | 0 | 0 |
| 170 | EN_KF_3.3 | DE | Kesselfeld | *Festuca rubra* aggr. | 71 | 0 | 0 |
| 171 | EN_KF_3.3 | DE | Kesselfeld | *Lolium perenne* | 71 | 0 | 0 |
| 172 | EN_KF_3.5 | DE | Kesselfeld | *Lolium perenne* | 69 | 0 | 0 |
| 173 | EN_KF_3.5 | DE | Kesselfeld | *Festulolium ×loliaceum* | 77 | 0 | 0 |
| 174 | EN_KF_4.1 | DE | Kesselfeld | *Festulolium ×loliaceum* | 77 | 0 | 0 |
| 175 | EN_KF_4.1 | DE | Kesselfeld | *Lolium perenne* | 69 | 0 | 0 |
| 176 | EN_KF_4.2 | DE | Kesselfeld | *Festulolium ×loliaceum* | 85 | 0 | 0 |
| 177 | EN_KF_4.3 | DE | Kesselfeld | *Festulolium ×loliaceum* | 87 | 0 | 0 |
| 178 | EN_KF_4.4 | DE | Kesselfeld | *Festulolium ×loliaceum* | 87 | 0 | 0 |
| 179 | EN_KF_4.4 | DE | Kesselfeld | *Lolium perenne* | 85 | 0 | 0 |
| 180 | EN_KF_4.5 | DE | Kesselfeld | *Festulolium ×loliaceum* | 93 | 0 | 0 |
| 181 | EN_KF_4.5 | DE | Kesselfeld | *Festuca rubra* aggr. | 29 | 0 | 0 |
| 182 | EN_KF_5.1 | DE | Kesselfeld | *Lolium perenne* | 83 | 0 | 0 |
| 183 | EN_KF_5.1 | DE | Kesselfeld | *Festulolium ×loliaceum* | 75 | 0 | 0 |
| 184 | EN_KF_5.2 | DE | Kesselfeld | *Lolium perenne* | 87 | 0 | 0 |
| 185 | EN_KF_5.2 | DE | Kesselfeld | *Festulolium ×loliaceum* | 75 | 0 | 0 |
| 186 | EN_KF_5.3 | DE | Kesselfeld | *Lolium perenne* | 85 | 0 | 0 |

| Sample ID | Plot | Country | Site | Grass species | Phenology | Lolitrem B (ng/g DW) | Ergovaline (ng/g DW) |
|---|---|---|---|---|---|---|---|
| 187 | EN_KF_5.3 | DE | Kesselfeld | *Festulolium ×loliaceum* | 89 | 0 | 0 |
| 188 | EN_KF_5.4 | DE | Kesselfeld | *Lolium perenne* | 85 | 43,78 | 0 |
| 189 | EN_KF_5.4 | DE | Kesselfeld | *Festulolium ×loliaceum* | 77 | 0 | 0 |
| 190 | EN_KF_5.5 | DE | Kesselfeld | *Festulolium ×loliaceum* | 77 | 0 | 0 |
| 191 | EN_KF_5.5 | DE | Kesselfeld | *Lolium perenne* | 87 | 0 | 0 |
| 61 | EN_DE_1.1 | DE | Iffeldorf | *Lolium perenne* | 61–65 | 0 | 0 |
| 62 | EN_DE_1.1 | DE | Iffeldorf | *Festuca rubra* aggr. | 61–65 | 0 | 0 |
| 69 | EN_DE_2.2 | DE | Iffeldorf | *Festuca rubra* aggr. | 69 | 0 | 0 |
| 70 | EN_DE_3.1 | DE | Iffeldorf | *Festuca rubra* aggr. | 61–65 | 0 | 0 |
| 71 | EN_DE_3.2 | DE | Iffeldorf | *Lolium perenne* | 61 | 0 | 0 |
| 72 | EN_DE_3.5 | DE | Iffeldorf | *Festulolium ×loliaceum* | 61 | 0 | 0 |
| 73 | EN_DE_4.3 | DE | Iffeldorf | *Lolium perenne* | 61–65 | 0 | 0 |
| 74 | EN_DE_4.4 | DE | Iffeldorf | *Festulolium ×loliaceum* | 65 | 0 | 0 |
| 75 | EN_DE_4.5 | DE | Iffeldorf | *Festuca rubra* aggr. | 61–65 | 0 | 0 |
| 76 | EN_DE_5.3 | DE | Iffeldorf | *Lolium perenne* | 29 | 0 | 0 |
| 77 | EN_DE_6.1 | DE | Iffeldorf | *Lolium perenne* | 71 | 0 | 0 |
| 78 | EN_DE_6.1 | DE | Iffeldorf | *Festuca rubra* aggr. | 71 | 0 | 106,9 |
| 79 | EN_DE_6.2 | DE | Iffeldorf | *Festuca rubra* aggr. | 75 | 0 | 0 |
| 80 | EN_DE_6.3 | DE | Iffeldorf | *Festuca rubra* aggr. | 73 | 0 | 69,48 |
| 81 | EN_DE_6.4 | DE | Iffeldorf | *Festuca rubra* aggr. | 81 | 0 | 23,36 |
| 82 | EN_DE_6.5 | DE | Iffeldorf | *Lolium multiflorum* | 69 | 0 | 0 |
| 83 | EN_DE_6.5 | DE | Iffeldorf | *Lolium perenne* | 73 | 0 | 0 |
| 84 | EN_DE_7.1 | DE | Iffeldorf | *Lolium perenne* | 77 | 0 | 0 |
| 85 | EN_DE_7.1 | DE | Iffeldorf | *Festuca rubra* aggr. | 87 | 0 | 0 |
| 86 | EN_DE_7.2 | DE | Iffeldorf | *Festuca rubra* aggr. | 93 | 0 | 0 |
| 87 | EN_DE_7.3 | DE | Iffeldorf | *Festuca rubra* aggr. | 29 | 0 | 0 |
| 88 | EN_DE_7.4 | DE | Iffeldorf | *Festuca rubra* aggr. | 29 | 0 | 0 |
| 89 | EN_DE_7.5 | DE | Iffeldorf | *Festuca rubra* aggr. | 29 | 0 | 0 |
| 90 | EN_DE_8.1 | DE | Iffeldorf | *Lolium perenne* | 85 | 0 | 0 |
| 91 | EN_DE_8.1 | DE | Iffeldorf | *Festuca rubra* aggr. | 73 | 0 | 0 |
| 92 | EN_DE_8.2 | DE | Iffeldorf | *Festuca rubra* aggr. | 75 | 0 | 39,01 |
| 93 | EN_DE_8.2 | DE | Iffeldorf | *Lolium perenne* | 81 | 0 | 0 |
| 94 | EN_DE_8.3 | DE | Iffeldorf | *Festulolium ×loliaceum* | 87 | 0 | 0 |
| 95 | EN_DE_8.3 | DE | Iffeldorf | *Lolium perenne* | 92 | 0 | 0 |
| 96 | EN_DE_8.4 | DE | Iffeldorf | *Lolium perenne* | 92 | 0 | 0 |
| 97 | EN_DE_8.5 | DE | Iffeldorf | *Lolium perenne* | 89 | 0 | 0 |
| 98 | EN_DE_9.1 | DE | Iffeldorf | *Lolium perenne* | 89 | 0 | 0 |
| 99 | EN_DE_9.2 | DE | Iffeldorf | *Lolium perenne* | 92 | 0 | 0 |
| 100 | EN_DE_9.3 | DE | Iffeldorf | *Lolium perenne* | 89 | 0 | 0 |
| 101 | EN_DE_9.4 | DE | Iffeldorf | *Lolium perenne* | 89 | 915,09 | 0 |
| 102 | EN_DE_9.5 | DE | Iffeldorf | *Lolium perenne* | 29 | 0 | 0 |
| 63 | EN_DE_10.1 | DE | Iffeldorf | *Lolium perenne* | 92 | 0 | 0 |
| 64 | EN_DE_10.2 | DE | Iffeldorf | *Lolium perenne* | 92 | 139,65 | 0 |
| 65 | EN_DE_10.3 | DE | Iffeldorf | *Lolium perenne* | 93 | 0 | 0 |
| 66 | EN_DE_10.4 | DE | Iffeldorf | *Lolium perenne* | 92 | 0 | 0 |
| 67 | EN_DE_10.5 | DE | Iffeldorf | *Festuca rubra* aggr. | 29 | 0 | 0 |
| 68 | EN_DE_10.5 | DE | Iffeldorf | *Lolium perenne* | 93 | 0 | 0 |
| 319 | EN_AT_1.3 | AT | Henndorf | *Lolium perenne* | NA | 0 | 0 |
| 325 | EN_AT_2.1 | AT | Henndorf | *Lolium perenne* | 59 | 0 | 0 |
| 326 | EN_AT_2.2 | AT | Henndorf | *Lolium perenne* | 59–61 | 0 | 0 |
| 327 | EN_AT_3.5 | AT | Henndorf | *Lolium perenne* | 61–65 | 0 | 0 |
| 328 | EN_AT_4.2 | AT | Henndorf | *Festuca rubra* aggr. | 29 + 99 | 0 | 0 |
| 329 | EN_AT_4.3 | AT | Henndorf | *Lolium perenne* | 29 + 99 | 0 | 0 |
| 330 | EN_AT_5.3 | AT | Henndorf | *Lolium perenne* | 61 | 0 | 0 |
| 331 | EN_AT_6.1 | AT | Henndorf | *Lolium multiflorum* | 73 | 0 | 2423 |
| 332 | EN_AT_6.2 | AT | Henndorf | *Lolium multiflorum* | 93 | 0 | 0 |
| 333 | EN_AT_6.2 | AT | Henndorf | *Lolium perenne* | 93 | 0 | 0 |
| 334 | EN_AT_6.3 | AT | Henndorf | *Festuca rubra* aggr. | 29 + 87 | 0 | 0 |
| 335 | EN_AT_6.4 | AT | Henndorf | *Lolium perenne* | 73 | 0 | 0 |
| 336 | EN_AT_6.4 | AT | Henndorf | *Lolium multiflorum* | 93 | 0 | 63 |
| 337 | EN_AT_6.5 | AT | Henndorf | *Lolium multiflorum* | 87 | 0 | 0 |
| 338 | EN_AT_6.5 | AT | Henndorf | *Lolium perenne* | 83 | 0 | 0 |
| 339 | EN_AT_7.1 | AT | Henndorf | *Lolium perenne* | 89 | 0 | 0 |
| 340 | EN_AT_7.1 | AT | Henndorf | *Lolium multiflorum* | 93 | 0 | 43,08 |
| 341 | EN_AT_7.2 | AT | Henndorf | *Lolium perenne* | 93 | 0 | 0 |
| 342 | EN_AT_7.3 | AT | Henndorf | *Festuca rubra* aggr. | 85 | 0 | 350,5 |
| 343 | EN_AT_7.3 | AT | Henndorf | *Lolium perenne* | 65 | 0 | 0 |
| 344 | EN_AT_7.4 | AT | Henndorf | *Lolium perenne* | 93 | 0 | 0 |
| 345 | EN_AT_7.4 | AT | Henndorf | *Festuca rubra* aggr. | 85 | 0 | 751,2 |

| Sample ID | Plot | Country | Site | Grass species | Phenology | Lolitrem B (ng/g DW) | Ergovaline (ng/g DW) |
|---|---|---|---|---|---|---|---|
| 346 | EN_AT_7.5 | AT | Henndorf | *Lolium multiflorum* | 85 | 0 | 3,09 |
| 347 | EN_AT_7.5 | AT | Henndorf | *Festuca rubra* aggr. | 85 | 0 | 8,49 |
| 348 | EN_AT_7.5 | AT | Henndorf | *Lolium perenne* | 83 | 0 | 0 |
| 349 | EN_AT_8.1 | AT | Henndorf | *Lolium multiflorum* | 65 | 0 | 0 |
| 350 | EN_AT_8.1 | AT | Henndorf | *Lolium perenne* | 61 | 0 | 0 |
| 351 | EN_AT_8.2 | AT | Henndorf | *Lolium multiflorum* | 65 | 0 | 0 |
| 352 | EN_AT_8.2 | AT | Henndorf | *Lolium perenne* | 61 | 0 | 0 |
| 353 | EN_AT_8.3 | AT | Henndorf | *Lolium multiflorum* | 65 | 0 | 0 |
| 354 | EN_AT_8.3 | AT | Henndorf | *Lolium perenne* | 61 | 0 | 0 |
| 355 | EN_AT_8.4 | AT | Henndorf | *Lolium perenne* | 69 | 0 | 0 |
| 356 | EN_AT_8.5 | AT | Henndorf | *Lolium perenne* | 69 | 0 | 0 |
| 357 | EN_AT_9.2 | AT | Henndorf | *Festuca rubra* aggr. | 83 | 0 | 0 |
| 358 | EN_AT_9.3 | AT | Henndorf | *Festuca rubra* aggr. | 83 | 0 | 0 |
| 359 | EN_AT_9.4 | AT | Henndorf | *Festuca rubra* aggr. | 87 | 0 | 0 |
| 360 | EN_AT_9.5 | AT | Henndorf | *Festuca rubra* aggr. | 87 | 0 | 0 |
| 320 | EN_AT_10.1 | AT | Henndorf | *Lolium perenne* | 89 | 0 | 0 |
| 321 | EN_AT_10.2 | AT | Henndorf | *Lolium perenne* | 92 | 0 | 0 |
| 322 | EN_AT_10.3 | AT | Henndorf | *Lolium multiflorum* | 87 | 0 | 0 |
| 323 | EN_AT_10.3 | AT | Henndorf | *Lolium perenne* | 89 | 0 | 29,34 |
| 324 | EN_AT_10.4 | AT | Henndorf | *Lolium perenne* | 89 | 0 | 6,73 |
| 361 | EN_HU_2.1 | HU | Szépalma | *Lolium perenne* | >93 | 0 | 0 |
| 362 | EN_HU_2.2 | HU | Szépalma | *Festuca rubra* aggr. | 61–65 | 0 | 0 |
| 363 | EN_HU_3.2 | HU | Szépalma | *Festuca rubra* aggr. | 65 | 0 | 0 |
| 364 | EN_HU_4.1 | HU | Szépalma | *Festuca rubra* aggr. | >93 | 74,54 | 560,19 |
| 365 | EN_HU_4.1 | HU | Szépalma | *Festuca rubra* aggr. | 65 | 0 | 0 |
| 366 | EN_HU_4.2 | HU | Szépalma | *Festuca rubra* aggr. | >93 | 0 | 285,14 |
| 367 | EN_HU_4.3 | HU | Szépalma | *Festuca rubra* aggr. | >93 | 0 | 2122,45 |
| 368 | EN_HU_4.3 | HU | Szépalma | *Festuca rubra* aggr. | >93 | 50,26 | 294,71 |
| 369 | EN_HU_4.5 | HU | Szépalma | *Festuca rubra* aggr. | >93 | 0 | 460,83 |
| 370 | EN_HU_5.1 | HU | Szépalma | *Festuca rubra* aggr. | 61 | 0 | 0 |
| 371 | EN_HU_5.2 | HU | Szépalma | *Festuca rubra* aggr. | 61 | 0 | 0 |
| 372 | EN_HU_5.3 | HU | Szépalma | *Festuca rubra* aggr. | 61 | 0 | 0 |
| 373 | EN_HU_5.5 | HU | Szépalma | *Festuca rubra* aggr. | 61 + 69 | 0 | 0 |
| 374 | EN_HU_5.5 | HU | Szépalma | *Festuca rubra* aggr. | 65 | 0 | 0 |

Seiler et al.: Environmental conditions and plant diversity show little effect on mycotoxin occurrence in European grasslands used for horse husbandry. – Tuexenia 45 (2025).

**Supplement E2.** Lab protocol for mycotoxin detection.

**Anhang E2.** Laborprotokoll zum Mykotoxin-Nachweis.

*Modified from a description provided by Dr. Gaétan Glauser from the Neuchâtel Platform of Analytical Chemistry, University of Neuchâtel, Switzerland*

Alkaloids were extracted from dried plant material as follows: first, 20 mg of tissue was ground to a fine powder using a mixer mill (Retsch MM400) and two UFO stainless steel beads (5.6 and 3.2 mm diameter). One mL of methanol:water:formic acid (70:30:0.1, v/v/v) was added, and the mixture was shaken in the mixer mill for 4 min at 30 Hz.

Following centrifugation, the supernatant was placed into an HPLC vial and 1 µL injected in a UPLC I-Class (Waters) system coupled to a QTRAP 6500+ (Sciex). The column for separation was an Acquity UPLC BEH C18 50mm x 2.1 mm internal diameter, 1.7 µm particle size (Waters). The mobile phases were (A) H2O + 0.05% formic acid and (B) acetonitrile + 0.05% formic acid. A gradient from 5% B to 93.2% B in 6.5 min at 0.4 mL/min was applied, followed by a hold at 100% B for 2 min and reequilibration at 5% B for 1.5 min. The column temperature was 25 °C.

Alkaloids were monitored in positive electrospray ionization using the following transitions: for ergovaline, quantitative (Q) and qualitative (q) transitions were 534.3→223.2 and 534.3→208.1, respectively. For lolitrem B, Q and q transitions were 686.4→238.2 and 686.4→628.4, respectively. Source parameters were IS 5500 V, TEM 400°C, GS1 50 psi, GS2 40 psi, and CUR 30 psi. The system was controlled by Analyst 1.7.1. Quantification was done by external calibration using reference standards of ergovaline and lolitrem B.

**Supplement E3.** Nationally red-listed species found in the plots.

**Anhang E3.** Vorkommende Arten der nationalen Roten Listen.

*NT – near threatened; VU – vulnerable; CR – critically endangered; * – status depending on subspecies (not identified)*

*NT – potenziell gefährdet; VU – gefährdet; CR – vom Aussterben bedroht; * – Status abhängig von der Unterart (nicht bestimmt).*

| Taxon | National Red-list status | Location (Country) | Year found |
|---|---|---|---|
| *Carex hostiana* | VU | Henndorf (AT) | 2022 |
| *Cirsium rivulare* | 3 (VU) | Iffeldorf (DE) | 2022 |
| *Cladium mariscus* | VU | Henndorf (AT) | 2022 |
| *Danthonia decumbens* | NT | Henndorf (AT) | 2022 |
| *Eleocharis uniglumis** | VU or CR | Henndorf (AT) | 2021 |
| *Helianthemum nummularium* | NT | Szépalma (HU) | 2023 |
| *Sanguisorba officinalis* | NT | Henndorf (AT)<br>Iffeldorf (DE)<br>Kesselfeld (DE) | 2022 |

**Supplement E4.** Rates of mycotoxin occurrence per site.

**Anhang E4.** Mykotoxin-Positivitätsraten pro Betrieb.

| | | Rate of occurrence (%) | | |
|---|---|---|---|---|
| **Site (country)** | **Number of samples tested** | **Total** | **Ergovaline** | **Lolitrem B** |
| Trévol (FR) | 126 | 31.7 | 11.9 | 27.8 |
| Braunenberg (DE) | 59 | 32.2 | 28.8 | 5.1 |
| Hermersberg (DE) | 52 | 26.9 | 23.1 | 11.5 |
| Kesselfeld (DE) | 37 | 5.4 | 2.7 | 2.7 |
| Iffeldorf (DE) | 42 | 14.3 | 9.5 | 4.8 |
| Henndorf (AT) | 42 | 21.4 | 21.4 | 0 |
| Szépalma (HU) | 14 | 35.7 | 35.7 | 14.3 |