

Management practice and soil properties affect plant productivity and root biomass in endophyte-symbiotic and endophyte-free meadow fescue grasses

Sanna Keronen¹ | Marjo Helander¹ | Kari Saikkonen² | Benjamin Fuchs² 

¹Department of Biology, University of Turku, Turku, Finland

²Biodiversity Unit, University of Turku, Turku, Finland

Correspondence

Benjamin Fuchs, Biodiversity Unit, University of Turku, Turku 20014, Finland.
Email: Benjamin.fuchs@utu.fi

Funding information

Suomen Kulttuurirahasto; Academy of Finland, Grant/Award Numbers: 311077, 324523, 326226

Abstract

Introduction: Pesticides are increasingly used in intensely managed agro-environments, with an increasingly acknowledged impact on crop production, root establishment and plant resilience. At the same time management practices are intensified with the goal to maximize productivity.

Materials and Methods: In a greenhouse, we studied the effects of three mowing regimes (uncut, and cutting 5 or 15 cm) employed three times during the season on root and shoot biomass and chlorophyll content of the cool-season grass *Festuca pratensis* (meadow fescue) growing in soil with a history of glyphosate-based herbicide (GBH) use, the corresponding control soil, and sterilized control soil. Half of the plants hosted a systemic and vertically transmitted fungal endophyte, *Epichloë uncinata*, which is known to promote host grass growth.

Results: Endophyte symbiosis did not affect any tested plant parameters. Cutting the plants to 5 cm decreased both root and cumulative shoot biomass. Herbicide soil history, together with intense cutting (5 cm), caused a decrease in shoot biomass and lowered the chlorophyll content. Surprisingly, soil sterilization boosted shoot biomass and chlorophyll concentrations during less intense cutting (15 cm) and noncutting when compared to the control soil. Root biomass reduced in uncut plants when growing in soil with a history of glyphosate use.

Conclusion: Our results indicate that GBH residues in the soil can diminish shoot biomass when grass is frequently cut. Decreased root biomass caused by soil glyphosate history goes together with a reduction of carbon allocation belowground, which decreases grassland resilience to climate warming, increasingly occurring droughts and extreme weather events.

KEYWORDS

agricultural practice, carbon sequestration, plant performance, resilience, soil microbes

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2022 The Authors. *Journal of Sustainable Agriculture and Environment* published by Global Initiative of Crop Microbiome and Sustainable Agriculture and John Wiley & Sons Australia, Ltd.

1 | INTRODUCTION

Empirical research over the past decades has demonstrated that plant productivity and resilience depend on soil quality and the abundance and diversity of soil microbes (Jacoby et al., 2017; Wagg et al., 2019; Wall et al., 2015). In addition, soil quality affects greenhouse gas emissions and water quality (Lal & Bruce, 1999; Skinner et al., 2019) and, thus, is linked to energy use in food production, food security, and the state of the environment (Muñoz-Rojas, 2018). Soil quality describes the capacity of soil to function as a provider of key ecosystem services, such as decomposing organic matter, supplying and cycling nutrients for optimum plant growth, receiving rainfall and storing water for root utilization, filtering water to support clean groundwater, storing organic carbon for nutrient retention, and mitigating greenhouse gas emissions (Franzluebbers, 2012; Muñoz-Rojas, 2018). All these soil properties are highly impacted by soil microbes (Fester et al., 2014).

Intensification of agricultural practices, such as tilling and the application of agrochemicals and artificial fertilizers, can reduce soil microbial diversity and multifunctionality (Tsiafouli et al., 2015; Wittwer et al., 2021). Microbial biomass and the abundance of beneficial root symbionts, such as arbuscular mycorrhizal fungi, are negatively linked to the amount of pesticide residue in the soil (Edlinger et al., 2022; Helander et al., 2019). As a consequence, pesticide residues are discussed as a key factor determining microbial soil life in agroecosystems, which determines plant productivity and stress resilience (Riedo et al., 2021).

In pastures and hay production, glyphosate-based herbicides (GBHs) are the most commonly used pesticides (Maggi et al., 2020). Glyphosate (N-[phosphonomethyl]-glycine) is a nonselective, systemic, post-emergence herbicide, and it acts as an inhibitor of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) in the shikimate pathway (Duke and Powles, 2008; Helander et al., 2012). The shikimate pathway exists in plants and in many microbes for the biosynthesis of essential aromatic amino acids (Gill et al., 2017; Leino et al., 2021). Glyphosate depletes the pool of compounds needed for carbon fixation, which causes a general disruption of an organism's metabolism (Duke & Powles, 2008) from phytochemical processes to carbon assimilation and translocation (Gomes et al., 2014). In addition to the effects caused by glyphosate directly, glyphosate residues and aminomethyl phosphonic acid (glyphosate's breakdown product) can indirectly affect the health of plants through changes in the rhizosphere microbiome (van Bruggen et al., 2018; Kremer & Means, 2009; Kuklinsky-Sobral et al., 2005). At the soil microbial level, glyphosate can reduce beneficial rhizosphere symbionts, including mycorrhizal fungi and nitrogen-fixing bacteria (van Bruggen et al., 2021; Helander et al., 2018; Mohamed et al., 2021; Ramula et al., 2021; Wilkes et al., 2020). Concentrations of pesticide residues in soil under conventional agriculture are ubiquitous, but residues are also found in organic agricultural management soils and may be introduced via animal manure fertilizer (Fuchs, Saikkonen, et al., 2022; Muola et al., 2021; Riedo et al., 2021). Glyphosate residues accumulate in agricultural soils worldwide (Maggi et al., 2020), but it remains to be fully elucidated how their antimicrobial properties affect soil microbes and

what their critical role is in plant resilience and ecosystem functions (Fuchs et al., 2021; Ruuskanen et al., 2023).

Soil degradation can be a result of intensive crop production, which is accompanied by a reduction in the soil's organic carbon (Bakker et al., 2018; Doetterl et al., 2016; Kirkels et al., 2014). Perennial grasses, mostly forage crops, have a high production of root biomass, and together with the absence or reduction of tillage, they regain organic soil matter and strengthen soil capacity for long-term productivity and environmental resilience (Franzluebbers, 2012; Jones & Donnelly, 2004; Paul et al., 2019; Ward et al., 2016). Consequently, well-managed grasslands can maintain and accumulate soil carbon and contribute to climate change mitigation (O'Mara, 2012; Poeplau et al., 2018). Grass is grown for its fodder quality and grazed or cut two to three times per year based on its regrowth capacity (O'Mara, 2012). Grass pastures are usually renewed every 2–3 years, and glyphosate is commonly used between renewal events.

We selected meadow fescue (*Schedonorus pratensis* [Huds.] P. Beauv. syn. *Festuca pratensis* [Huds.] and *Lolium pratense* [Huds.]) as a model species due to its importance as a forage grass in temperate and cold climates. It is perennial with a height of 40–100 cm. It grows in a tuft formation and has rapid growth in spring, high regrowth ability, and low straw formation. Meadow fescue has excellent winter hardiness and forage quality (Brink et al., 2010; Fjellheim & Rognli, 2005; Saari et al., 2010). Meadow fescue is a viable alternative to tall fescue (*Schedonorus arundinacea*) and orchard grass (*Dactylis glomerata*) in managed intensive rotational grazing systems because of its comparable yield and superior fibre digestibility. Meadow fescue has a greater nutritional quality than, for example, tall fescue or orchard grass (Brink et al., 2010).

Meadow fescue can be symbiotic with the systematic fungal endophyte *Epichloë uncinata* ([W. Gams, Petrini, and D. Schmidt] Leuchtm. and Scharld syn. *Neotyphodium uncinatum*). The endophyte subsists entirely on the host's resources, and its fitness is tightly linked to the fitness of the host plant due to strictly vertical transmission (Saikkonen et al., 2004). Meadow fescue benefits from the endophyte via increased growth and reproduction, and resistance to herbivores, pathogens, and abiotic environmental stresses, which thereby enhances the competitive abilities of endophyte-infected plants (Fuchs & Krauss, 2019; Saikkonen et al., 2004). The symbiosis between *E. uncinata* and meadow fescue can range from antagonistic to mutualistic depending on the genetic match of the fungal endophyte and the host grass and environmental conditions (Ahlholm et al., 2002; Saikkonen, 2004; Saikkonen et al., 2010). The endophyte is most beneficial to host grass in high nutrient environments (e.g., in agricultural fields) and can become costly under low nutrient conditions, decreasing plant performance (Cheplick & Faeth, 2009; Saikkonen et al., 2013, 2006).

Here, we tested whether long-term (8-year) soil treatment with GBHs affects meadow fescue biomass (root and shoot), regrowth, and chlorophyll content under three different cutting practices and whether the effects can be explained by a lack of soil microbes. As suggested in several studies, glyphosate is an antimicrobial agent which is likely to alter the soil and rhizosphere microbiome (reviewed in Ruuskanen et al., 2023). Consequently, our study looks into plant

responses to the effect of long-term, field realistic GBH application as a treatment, which includes potential indirect GBH-mediated changes in soil microbiome and soil biogeochemistry. We hypothesize that (1) GBH history in soil decreases overall plant biomass and chlorophyll content and that the effect is most pronounced under extensive cutting, and (2) GBH history in soil yields results similar to sterilized soil mediated by a potential reduction of beneficial soil microbes.

2 | MATERIALS AND METHODS

2.1 | Study setup

Meadow fescue seeds with known endophyte status of systemic and vertically transmitted *Epichloë uncinata* symbiosis were collected from plants growing in an experimental field at the Ruissalo Botanical Garden in Turku in the fall of 2020. In 2021, randomly chosen seeds from each plant were checked for the presence (E+) and absence (E-) of endophytic mycelium via staining with aniline blue followed by light microscopy where the endophytic hyphae is visible in seeds of E+ plants. Seeds from seven E- and E+ grasses were separately pooled and sowed in 1.5 L pots in the following three soil treatments: soil with a GBH treatment history (GBH), control soil (C), and sterilized soil (S) (30 E- and 30 E+ plants × 3 soil treatments = 180 pots total). To mimic the harvest practices of fodder grasses, 10 plants per treatment combination (soil type × endophyte) were assigned either to 5 or 15 cm cutting or remained uncut until the biomass was harvested. Grasses were cut on 18 June 2021, 9 July 2021 and 5 August 2021, and harvested on 6–10 September 2021. Plants were arranged in a greenhouse according to the randomized block design and grown with ambient light and temperature (20–26°C) from mid-May until mid-September.

2.2 | Soil treatment

Soil was collected from a long-term field experiment established in 2013 at the Botanical Garden of Ruissalo (Helander et al., 2019). The soil in the field is a medium clay enhanced with sand and peat. The nutrient values of the soil were (based on an analysis made in 2016): pH 6.2, phosphorus 4.2 mg/l, potassium 250 mg/l, calcium 1900 mg/l, magnesium 570 mg/l, sulphur 10.6 mg/l, zinc 2.74 mg/l, copper 7.5 mg/l, and manganese 15 mg/l. In spring 2014, the field (25 × 50 m) was divided into alternating ten control (C) and 10 GBH strips (1.5 × 23 m) with 1.5 m buffer strips between them. Since then, the C strips were treated with tap water (5 l/strip) and GBH strips (GBH) with Roundup Gold® (450 g/l isopropylamine glyphosate salt, application rate: 6.4 l/ha) twice per year (in the spring and the fall), which is a common method in no-till practice. The experimental setup is unique as it provides soil without GBH contamination as well as soil from the same field with a long-term history of GBH use. Regular analyses confirmed the exclusion of glyphosate drift into control plots. After treatment of the soil in spring, the exact same number of various crop species were planted on treated and untreated strips, and all strips were hand weeded throughout the season. Consequently, all vegetation

on all strips is the same, which is the adequate setting to minimize cofactors and focus on the effects of GBH use and history. The soil for the experimental pots was collected from the GBH and control (C) strips 2 weeks after treatment in spring before crop plants were planted. Sterilized soil (S) was prepared by heat-sterilizing soil collected from C strips in an autoclave (120°C for 20 min).

2.3 | Chlorophyll measurements

Before the final biomass measurement, 0.5 cm leaf discs were taken from the central part of one leaf blade per plant. Leaf collection was standardized by choosing the leaf of which position (starting from the top) was closest to the total leaf number divided by two (e.g., the fifth leaf of a plant with 10 leaves and the third leaf of a plant with six leaves). The leaf disk was placed in a 2 ml reaction tube (Eppendorf GmbH) with 1 ml of N,N-dimethylformamide. Then, chlorophyll was extracted at room temperature in the dark for 24 h. Leaf disks were removed from the tubes before centrifugation at 12,000 rpm for 5 min. Then, 750 µl of the remaining liquid was transferred into a cuvette and analyzed with a Shimadzu UV-1900 UV-VIS spectrophotometer (Japan) by measuring absorbance at wavelengths of 663.8, 646.8, and 750 nm. Quantification of chlorophyll *a* and *b* content was done using equations in Porra et al. (1989).

2.4 | Plant biomass quantification

After each of the three cutting treatment events and after the final harvest, fresh green biomass was weighed, dried (at 65°C for 48 h in an oven), and weighed again. To estimate the yearly cumulative yield (shoot biomass), we summed the biomass of each cutting event for each plant. During the final harvest, the roots were carefully separated from the soil by gently rinsing them in water until they were clean. After washing, the roots were dried in an oven (at 65°C for 48 h) and weighed.

2.5 | Statistics

We tested the effects of cutting height, endophyte status, and soil treatment, as well as their interaction with total aboveground biomass, root biomass, and chlorophyll content, via an analysis of variance (ANOVA), before applying Tukey's post hoc test comparing single treatments. Plant root:shoot ratios were analyzed using ANOVA and Tukey post-hoc tests to compare each clipping treatment. Statistical analyses (ANOVA and correlation) were performed using R.

3 | RESULTS

All plants except for five grasses in GBH-treated soil and one grass in control soil survived until the end of the experiment. Endophyte symbiosis, neither alone nor in interaction with any of the clipping

and soil treatments, affected any of the plant response variables. Consequently, endophyte status was removed from the statistical model as explanatory variable.

3.1 | Shoot biomass

Uncut meadow fescues produced the largest cumulative shoot biomass (added biomass from three clipping events), which was significantly higher compared to shoot biomass from grasses cut to 15 or 5 cm (Figure 1, Tables 1 and 2). Grasses cut to a height of 5 cm had the smallest shoot biomass, which was significantly smaller than grasses cut to 15 cm (Figure 1, Tables 1 and 2). Plants grown in sterilized soil produced greater shoot biomass than those in the control and GBH-treated soils in the case of uncut plants and plants cut to 15 cm (Figure 1, Tables 1 and 3). In

contrast, in the case of plants cut to 5 cm, there was no statistically significant difference in shoot biomass between the control and sterilized soil treatments, but plants growing in GBH-treated soil produced less shoot biomass (Figure 1, Tables 1 and 3).

3.2 | Root biomass

Root biomass was strongly affected by clipping treatment; the more the grass was cut, the larger the reduction in biomass accumulation (Figure 2, Tables 1 and 2). Root biomass did not differ significantly between the soil treatments within each cutting regime (5 and 15 cm) compared to control soil (Figure 2, Tables 1 and 2). However, root biomass in uncut plants was lower for plants grown in GBH-treated soil compared to the control and sterilized soils (Figure 2, Tables 1 and 3).

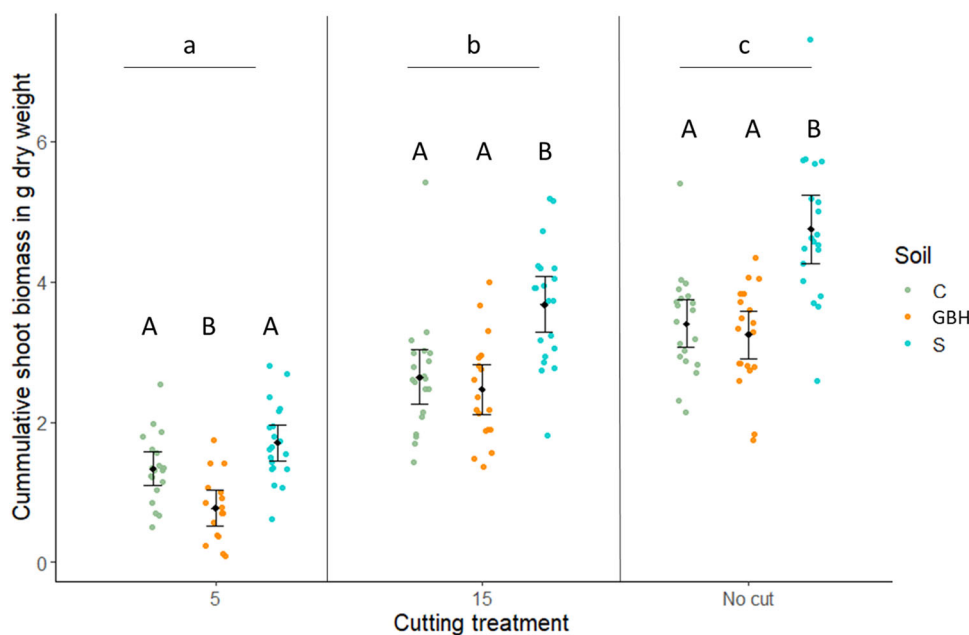


FIGURE 1 Cumulative aboveground dry biomass (shoot biomass) of meadow fescue grown in different soil treatments (C = control, GBH = GBH-treated, S = sterilized) and cut to a height of 5 or 15 cm thrice per season, or left uncut, as indicated. Data points show individual measurements, and error bars show the mean values \pm standard error (SE). Lowercase letters indicate a significant difference between aboveground biomasses in response to cutting treatment (Tukey pairwise comparison test). Uppercase letters indicate significant differences in response to soil treatment within each cutting category (Tukey pairwise comparison test) ($N = 156$).

Effector variable	df	Shoot biomass		Root biomass		Root:shoot ratio		Chlorophyll ($\mu\text{g}/\text{cm}^2$)	
		F	p	F	p	F	p	F	p
Cutting	2165	166.3	<0.001	207.41	<0.001	111.34	<0.001	9.78	<0.001
Soil	2165	42.87	<0.001	10.41	<0.001	3.49	0.033	7.25	<0.001
Cutting \times soil	4165	2.284	0.063	6.69	<0.001	4.82	0.001	3.10	0.017

Note: The analysis of variance summary table with p values and degrees of freedom is shown. Statistically significant values are marked bold.

TABLE 1 Shoot biomass, root biomass, root:shoot ratio and chlorophyll content were analyzed with a linear model testing the effector variables cutting (three levels), soil (three levels), and their interaction terms

TABLE 2 Summary table from a Tukey post-hoc test analyzing the plant responses to cutting treatment independent of soil treatment

Pairwise comparison	Shoot biomass		Root biomass		Root:shoot ratio		Chlorophyll ($\mu\text{g}/\text{g}/\text{cm}^2$)	
	t	p	t	p	t	p	t	p
5 versus 15 cm	9.524	<0.001	4.92	<0.001	3.00	0.008	3.67	0.001
No cut versus 15 cm	5.149	<0.001	12.88	<0.001	10.51	<0.001	0.08	0.990
No cut versus 5 cm	14.62	<0.001	17.59	<0.001	13.34	<0.001	3.61	0.001

Note: Statistically significant values are marked bold.

TABLE 3 Summary table from a Tukey post hoc test analyzing the soil treatment within each cutting treatment

Pairwise comparison	Shoot biomass		Root biomass		Root:shoot ratio		Chlorophyll ($\mu\text{g}/\text{g}/\text{cm}^2$)		
	t	p	t	p	t	p	t	p	
5 cm	C versus GBH	3.23	<0.005	2.63	0.056	1.97	0.131	2.64	0.029
	C versus S	2.28	0.068	0.19	0.981	0.94	0.619	0.36	0.930
	S versus GBH	5.45	<0.001	2.21	0.079	2.88	0.016	2.32	0.062
15 cm	C versus GBH	0.70	0.767	0.45	0.894	0.06	0.998	0.52	0.860
	C versus S	4.02	<0.001	0.05	0.999	1.62	0.246	0.75	0.732
	S versus GBH	4.67	<0.001	0.40	0.915	1.65	0.232	1.27	0.419
No cut	C versus GBH	0.59	0.828	2.85	0.017	3.42	0.003	1.24	0.437
	C versus S	5.09	<0.001	1.69	0.219	1.81	0.177	4.36	<0.001
	S versus GBH	5.67	<0.001	4.54	<0.001	1.61	0.248	3.13	0.008

Note: Statistically significant values are marked bold.

3.3 | Root:shoot ratio

The ratio of root biomass to shoot (aboveground) biomass was significantly affected by the clipping treatment, showing a smaller root:shoot ratio following the extent of cutting (Figure 3, Tables 1 and 2). Soil treatment affected the root:shoot ratio differently depending on the cutting treatment. In plants cut to 5 cm, GBH-treated soil caused an increase in the root:shoot ratio compared to sterilized soil, whereas in uncut plants, GBH-treated soil caused a reduction in the root:shoot ratio compared to the control soil (Figure 3, Tables 1 and 3).

3.4 | Chlorophyll content

Chlorophyll content was reduced by the most intense cutting treatment (5 cm) compared to the control and less intense cutting (15 cm) (Figure 4, Tables 1 and 2). Grasses grown in GBH-treated soil had reduced chlorophyll content in plants cut to 5 cm, showing a similar pattern to shoot biomass (Figures 1 and 4). Sterilized soil caused an increase in chlorophyll content compared to the control soil and GBH-treated soil when plants were never cut (Figure 4, Tables 1 and 3), again showing a similar pattern to shoot biomass (Figure 1).

4 | DISCUSSION

Grasslands represent approximately 40% of the earth's terrestrial ecosystems and 70% of its agricultural area (Hopkins & Holz, 2006; Michalk et al., 2013; Ramankutty et al., 2008). Thus, grasslands support dairy-and meat-based food production, provide habitats for biodiversity, and deliver numerous ecosystem services (Bai & Cotrufo, 2022; Franzluebbers, 2012). Because many perennial grasses have high organic matter content and deep root systems, grasslands facilitate nutrient cycling, mitigate greenhouse gas emissions through soil carbon sequestration, and are used to prevent erosion via the mechanical reinforcement of soil.

Our results suggest that the productivity and tolerance of forage grass can be either increased or decreased depending on the extent of agricultural practices. Extensive mowing of meadow fescue plants (cutting to 5 cm) led to poor regrowth rates, decreased chlorophyll concentrations, and reduced shoot biomass throughout the season. Furthermore, root biomass was reduced by cutting, indicating that atmospheric carbon was not optimally sequestered in intensely managed hay fields (Eze et al., 2018; Law et al., 2016). In particular, extensive cutting (5 cm) causes small root biomass, which can lead to decreased belowground carbon sequestration, decreased plant stress resilience, and increased soil erosion (Bardgett et al., 2021; Franzluebbers, 2012; Mchunu & Chaplot, 2012).

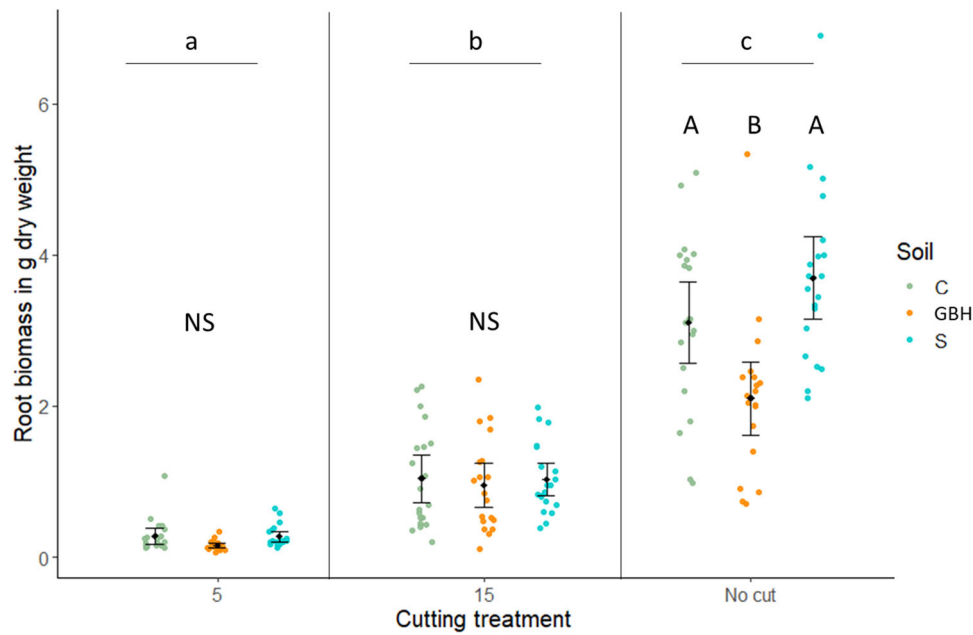


FIGURE 2 Root dry biomass of meadow fescue grown in different soil treatments (C = control, GBH = GBH-treated, S = sterilized) and cut to a height of 5 or 15 cm thrice per season, or left uncut, as indicated. Data points show individual measurements, and error bars show the mean values \pm SE. Lowercase letters indicate a significant difference between root biomass in response to cutting treatment (Tukey pairwise comparison test). Uppercase letters indicate significant differences in response to soil treatment within each cutting category (Tukey pairwise comparison test) ($N = 156$).

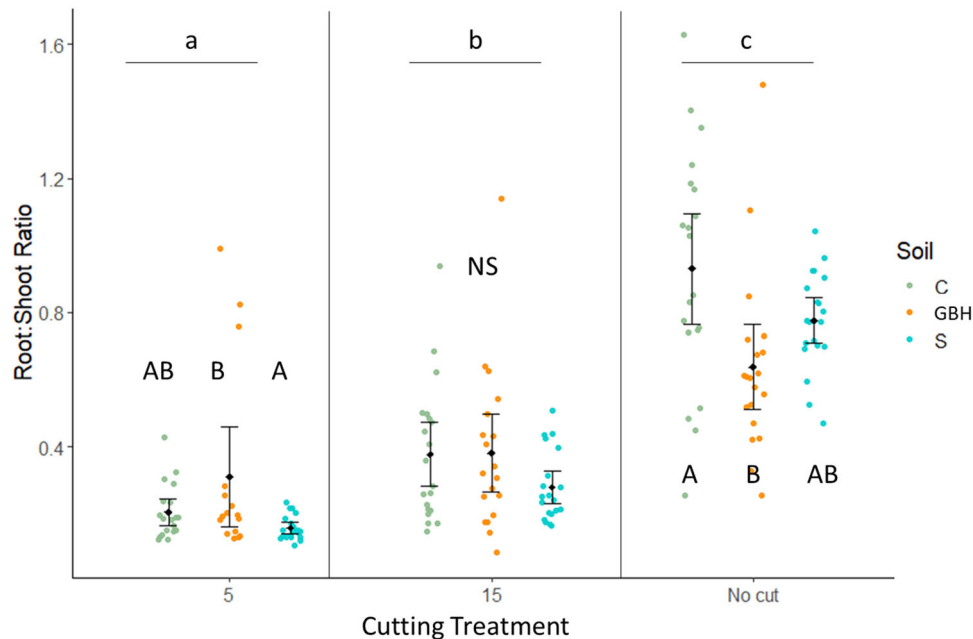


FIGURE 3 Root:shoot ratio calculated as root dry biomass divided by aboveground dry biomass. Root:shoot ratio of meadow fescue grown in different soil treatments (C = control, GBH = GBH-treated, S = sterilized) and cut to a height of 5 or 15 cm thrice per season, or left uncut, as indicated. Data points show individual measurements, and error bars show the mean values \pm SE. Lowercase letters indicate a significant difference between root:shoot ratio in response to cutting treatment (Tukey pairwise comparison test). Uppercase letters indicate significant differences in response to soil treatment within each cutting category (Tukey pairwise comparison test) ($N = 156$).

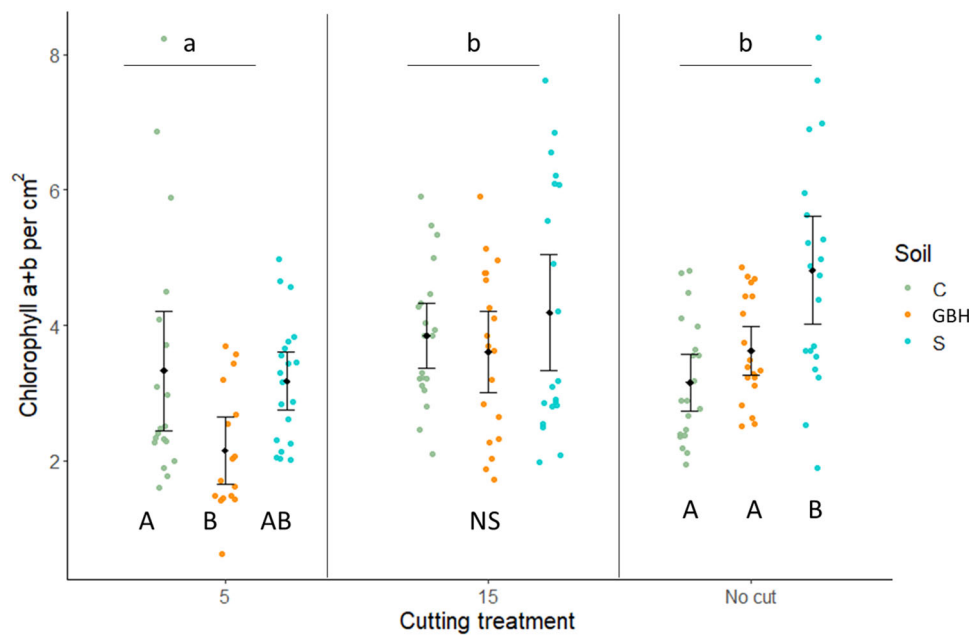


FIGURE 4 Chlorophyll content ($a + b$ per cm^2) of meadow fescue grown in different soil treatments (C = control, GBH = GBH-treated, S = sterilized) and cut to a height of 5 or 15 cm thrice per season, or left uncut, as indicated. Data points show individual measurements, and error bars show the mean values \pm SE. Lowercase letters indicate a significant difference between chlorophyll content in response to cutting treatment (Tukey pairwise comparison test). Uppercase letters indicate significant differences in response to soil treatment within each cutting category (Tukey pairwise comparison test) ($N = 156$).

Furthermore, our results demonstrate that soils with a history of herbicide use caused a reduction in total shoot biomass and chlorophyll concentration when the grass was under intense cutting (5 cm), demonstrating reduced regrowth ability. Contrary to previous studies (Muola et al., 2021), glyphosate residues in soil did not, however, significantly reduce the plant biomass of uncut meadow fescue grasses. Uncut plants showed a reduction in root biomass when grown in GBH-treated soil, demonstrating the negative effect of herbicide legacy in soil on root development, which may affect plants' responses to additional stressors under field conditions (Fuchs, Saikkonen, et al., 2022). Root:shoot ratio analyses showed that a small root system can result in relatively large plants. Cutting decreased the root:shoot ratio, with the most intense cutting resulting in the smallest ratio and the uncut treatment resulting in the largest ratio. Within uncut plants, soil herbicide history resulted in a reduced root:shoot ratio, indicating that larger shoot biomass was achieved with smaller root biomass. An explanation for this might be that GBHs add phosphate to the soil, which can serve as plant fertilizer (Hébert et al., 2019). It is noteworthy that our study was performed under greenhouse conditions with regular watering, and the growth diminishing properties of GBH residues in soil may become apparent only under stress conditions. However, taken together, these results indicate that soil herbicide history primarily affects root biology, which can affect soil carbon sequestration and regrowth of plants under co-occurring stressors, such as cutting (Bai & Cotrufo, 2022; Eze et al., 2018; Mchunu & Chaplot, 2012).

We suspected that a GBH-mediated reduction in root-associated soil microbes would be a driver of reduced plant performance.

However, our results indicate that the elimination of soil microorganisms by sterilization does not inhibit grass performance. In contrast, grasses performed better in sterilized soil compared to control soil. Plants grown in sterilized soil showed greater shoot growth and chlorophyll content compared to grasses growing in the control or GBH-treated soil.

Hines et al. (2017) demonstrate that grasses were mostly unaffected by alteration in soil microbiota but highly affected by nutrient status of the soil in comparison to forbs. That indicates that grasses are largely independent of root-associated microbes for nutrient uptake. Consequently, high nutrient sterilized soil excluded potential harmful microbes (van der Putten & Peters, 1997) but delivered ad libitum nutrient for an optimal growth. Regardless of the causes, these results suggest that reduced plant performance mediated by soil herbicide history is not strongly based on the reduction of soil or root-associated plant mutualistic microbes (Edlinger et al., 2022; Helander et al., 2019) but may be a result of disrupted plant homeostasis affecting growth and biomass accumulation or decreased nutrient accumulation (Bott et al., 2008; Fuchs, Laihonon, et al., 2022; Zobiolo et al., 2012).

Similar to soil and root-associated microbes, and contrary to past studies suggesting that *Epichloë* grass endophytes may promote environmentally friendly crop productivity by increasing host plant growth and reproduction (Cheplick & Faeth, 2009; Kauppinen et al., 2016; Saikkonen et al., 2004), endophyte status did not significantly affect the total aboveground plant biomass, root biomass, or chlorophyll content of meadow fescue in this study. One explanation for a missing effect in the clipped plants may be the induction of nitrogen rich alkaloids

due to clipping, which may come at the cost of improving biomass (Bultman et al., 2004; Fuchs et al., 2017a). However, if that was the case we should have recorded increased biomass in endophyte-symbiotic uncut plants, which was not the case. Another reason may be the age of the plants or cultivar specific performance. Some studies have indicated that the beneficial effect of endophytes is frequently missing during the first year of plant establishment or highly strain specific (Fuchs et al., 2017b, 2020; König et al., 2018).

5 | CONCLUSION

Our study demonstrates that mowing practices can determine the biomass of grasses above and below ground. Grazing or cutting close to the ground level can inhibit grass recovery, which results in a lower root:shoot ratio and indicates a decrease in root-based carbon sequestration. During interactions with additional stressors, such as long-term herbicide use, plant biomass productivity is inhibited above or below ground, which counteracts sustainable development goals in agricultural practices. In our study, grasses displayed optimized growth in the absence of soil microbes under greenhouse conditions. Under field conditions, results may vary and require deeper investigation. Future studies need to elucidate how long-term herbicide use affects the soil microbiome and cascading effects on crop plants, particularly in species such as legumes, which are highly dependent on root-associated microbes for nutrient acquisition.

AUTHOR CONTRIBUTIONS

Sanna Keronen, Marjo Helander, Kari Saikkonen and Benjamin Fuchs designed the experiment. Sanna Keronen and Benjamin Fuchs performed experiments and collected data. Sanna Keronen and Benjamin Fuchs analyzed the data. Sanna Keronen and Benjamin Fuchs wrote the manuscript with significant input from all co-authors. Marjo Helander, Kari Saikkonen and Benjamin Fuchs secured funding and provided guidance to undertake the work. All authors provided feedback on the manuscript.

ACKNOWLEDGEMENTS

We thank Esa Tyystjärvi and Heta Mattila for their help with the chlorophyll analyses. We thank Academy of Finland (B. F., Grant No. 324523; K. S., Grant Nos. 326226; M. H., Grant No. 311077) and Finnish Cultural Foundation (K. S., B. F.) for financial support.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The authors declare that they followed the ethics policies of the journal.

ORCID

Benjamin Fuchs  <http://orcid.org/0000-0002-3678-3284>

REFERENCES

- Ahlholm JU, Helander M, Lehtimäki S, Wäli P, Saikkonen K. Vertically transmitted fungal endophytes: different responses of host-parasite systems to environmental conditions. *Oikos*. 2002;99:173–83.
- Bai Y, Cotrufo MF. Grassland soil carbon sequestration: current understanding, challenges, and solutions. *Science*. 2022;377:603–8. <https://doi.org/10.1126/science.abo2380>
- Bakker PAHM, Pieterse CMJ, de Jonge R, Berendsen RL. The soil-borne legacy. *Cell*. 2018;172:1178–80. <https://doi.org/10.1016/j.cell.2018.02.024>
- Bardgett RD, Bullock JM, Lavorel S, Manning P, Schaffner U, Ostle N, et al. Combatting global grassland degradation. *Nat Rev Earth Environ*. 2021;2:720–35. <https://doi.org/10.1038/s43017-021-00207-2>
- Bott S, Tesfamariam T, Candan H, Cakmak I, Römheld V, Neumann G. Glyphosate-induced impairment of plant growth and micronutrient status in glyphosate-resistant soybean (*Glycine max* L.). *Plant Soil*. 2008;312:185–94. <https://doi.org/10.1007/s11104-008-9760-8>
- Brink GE, Casler MD, Martin NP. Meadow fescue, tall fescue, and orchardgrass response to defoliation management. *Agron J*. 2010;102:667–74. <https://doi.org/10.2134/agronj2009.0376>
- van Bruggen AHC, Finckh MR, He M, Ritsema CJ, Harkes P, Knuth D, et al. Indirect effects of the herbicide glyphosate on plant, animal and human health through its effects on microbial communities. *Front Environ Sci*. 2021;9:464.
- van Bruggen AHC, He MM, Shin K, Mai V, Jeong KC, Finckh MR, et al. Environmental and health effects of the herbicide glyphosate. *Sci Total Environ*. 2018;616–617:255–68. <https://doi.org/10.1016/j.scitotenv.2017.10.309>
- Bultman TL, Bell G, Martin WD. A fungal endophyte mediates reversal of wound-induced resistance and constrains tolerance in a grass. *Ecology*. 2004;85:679–85. <https://doi.org/10.1890/03-0073>
- Cheplick GP, Faeth SH. Ecology and evolution of the grass-endophyte symbiosis. USA: Oxford University Press; 2009.
- Doetterl S, Berhe AA, Nadeu E, Wang Z, Sommer M, Fiener P. Erosion, deposition and soil carbon: a review of process-level controls, experimental tools and models to address C cycling in dynamic landscapes. *Earth Sci Rev*. 2016;154:102–22. <https://doi.org/10.1016/j.earscirev.2015.12.005>
- Duke SO, Powles SB. Glyphosate: a once-in-a-century herbicide. *Pest Manag Sci*. 2008;64:319–25. <https://doi.org/10.1002/ps.1518>
- Edlinger A, Garland G, Hartman K, Banerjee S, Degrune F, García-Palacios P, et al. Agricultural management and pesticide use reduce the functioning of beneficial plant symbionts. *Nat Ecol Evol*. 2022;6:1145–54. <https://doi.org/10.1038/s41559-022-01799-8>
- Eze S, Palmer SM, Chapman PJ. Soil organic carbon stock in grasslands: effects of inorganic fertilizers, liming and grazing in different climate settings. *J Environ Manage*. 2018;223:74–84. <https://doi.org/10.1016/j.jenvman.2018.06.013>
- Fester T, Giebler J, Wick LY, Schlosser D, Kästner M. Plant-microbe interactions as drivers of ecosystem functions relevant for the biodegradation of organic contaminants. *Curr Opin Biotechnol*. 2014;27:168–75. <https://doi.org/10.1016/j.copbio.2014.01.017>
- Fjellheim S, Rognli O-A. Genetic diversity within and among Nordic Meadow Fescue (*Festuca pratensis* Huds.) Cultivars determined on the basis of AFLP markers. *Crop Sci*. 2005;45:2081–6. <https://doi.org/10.2135/cropsci2005.0091>
- Franzluebbers AJ. Grass roots of soil carbon sequestration. *Carbon Manag*. 2012;3:9–11. <https://doi.org/10.4155/cmt.11.73>
- Fuchs B, Krauss J. Can Epichloë endophytes enhance direct and indirect plant defence? *Fungal Ecol*. 2019;38:98–103. <https://doi.org/10.1016/j.funeco.2018.07.002>

- Fuchs B, Krischke M, Mueller MJ, Krauss J. Herbivore-specific induction of defence metabolites in a grass–endophyte association. *Funct Ecol.* 2017a;31:318–24. <https://doi.org/10.1111/1365-2435.12755>
- Fuchs B, Krischke M, Mueller MJ, Krauss J. Plant age and seasonal timing determine endophyte growth and alkaloid biosynthesis. *Fungal Ecol.* 2017b;29:52–8. <https://doi.org/10.1016/j.funeco.2017.06.003>
- Fuchs B, Kuhnert E, Krauss J. Contrasting effects of grass - endophyte chemotypes on a tri-trophic cascade. *J Chem Ecol.* 2020;46:422–9. <https://doi.org/10.1007/s10886-020-01163-9>
- Fuchs B, Laihonon M, Muola A, Saikkonen K, Dobrev PI, Vankova R, et al. A glyphosate-based herbicide in soil differentially affects hormonal homeostasis and performance of non-target crop plants. *Front Plant Sci.* 2022;12:787958.
- Fuchs B, Saikkonen K, Helander M. Glyphosate-modulated biosynthesis driving plant defense and species interactions. *Trends Plant Sci.* 2021;26:312–23. <https://doi.org/10.1016/j.tplants.2020.11.004>
- Fuchs B, Saikkonen K, Helander M, Tian Y, Yang B, Engström MT, et al. Legacy of agrochemicals in the circular food economy: glyphosate-based herbicides introduced via manure fertilizer affect the yield and biochemistry of perennial crop plants during the following year. *Chemosphere.* 2022;308:136366. <https://doi.org/10.1016/j.chemosphere.2022.136366>
- Gill JPK, Sethi N, Mohan A. Analysis of the glyphosate herbicide in water, soil and food using derivatising agents. *Environ Chem Lett.* 2017;15: 85–100. <https://doi.org/10.1007/s10311-016-0585-z>
- Gomes MP, Smedbol E, Chalifour A, Hénault-Ethier L, Labrecque M, Lepage L, et al. Alteration of plant physiology by glyphosate and its by-product aminomethylphosphonic acid: an overview. *J Exp Bot.* 2014;65:4691–4703. <https://doi.org/10.1093/jxb/eru269>
- Hébert M-P, Fugère V, Gonzalez A. The overlooked impact of rising glyphosate use on phosphorus loading in agricultural watersheds. *Front Ecol Environ.* 2019;17:48–56. <https://doi.org/10.1002/fee.1985>
- Helander M, Pauna A, Saikkonen K, Saloniemi I. Glyphosate residues in soil affect crop plant germination and growth. *Sci Rep.* 2019;9: 19653. <https://doi.org/10.1038/s41598-019-56195-3>
- Helander M, Saloniemi I, Omacini M, Druille M, Salminen J-P, Saikkonen K. Glyphosate decreases mycorrhizal colonization and affects plant-soil feedback. *Sci Total Environ.* 2018;642:285–91. <https://doi.org/10.1016/j.scitotenv.2018.05.377>
- Helander M, Saloniemi I, Saikkonen K. Glyphosate in northern ecosystems. *Trends Plant Sci.* 2012;17:569–74. <https://doi.org/10.1016/j.tplants.2012.05.008>
- Hines J, Pabst S, Mueller KE, Blumenthal DM, Cesarz S, Eisenhauer N. Soil-mediated effects of global change on plant communities depend on plant growth form. *Ecosphere.* 2017;8:e01996. <https://doi.org/10.1002/ecs2.1996>
- Hopkins A, Holz B. Grassland for agriculture and nature conservation: production, quality and multi-functionality. *Agron Res.* 2006;4: 3–20.
- Jacoby R, Peukert M, Succuro A, Koprivova A, Kopriva S. The role of soil microorganisms in plant mineral nutrition—current knowledge and future directions. *Front Plant Sci.* 2017;8:1617.
- Jones MB, Donnelly A. Carbon sequestration in temperate grassland ecosystems and the influence of management, climate and elevated CO₂. *New Phytol.* 2004;164:423–39. <https://doi.org/10.1111/j.1469-8137.2004.01201.x>
- Kauppinen M, Saikkonen K, Helander M, Pirttilä AM, Wäli PR. Epichloë grass endophytes in sustainable agriculture. *Nat Plants.* 2016;2:15224. <https://doi.org/10.1038/nplants.2015.224>
- Kirkels FMSA, Cammeraat LH, Kuhn NJ. The fate of soil organic carbon upon erosion, transport and deposition in agricultural landscapes—a review of different concepts. *Geomorphology.* 2014;226:94–105. <https://doi.org/10.1016/j.geomorph.2014.07.023>
- König J, Fuchs B, Krischke M, Mueller MJ, Krauss J. Hide and seek—infection rates and alkaloid concentrations of *Epichloë festucae* var. *lolii* in *Lolium perenne* along a land-use gradient in Germany. *Grass Forage Sci.* 2018;73:510–6. <https://doi.org/10.1111/gfs.12330>
- Kremer RJ, Means NE. Glyphosate and glyphosate-resistant crop interactions with rhizosphere microorganisms. *Eur J Agron.* 2009;31: 153–61. <https://doi.org/10.1016/j.eja.2009.06.004>
- Kuklinsky-Sobral J, Araújo WL, Mendes R, Pizzirani-Kleiner AA, Azevedo JL. Isolation and characterization of endophytic bacteria from soybean (*Glycine max*) grown in soil treated with glyphosate herbicide. *Plant Soil.* 2005;273:91–9. <https://doi.org/10.1007/s11104-004-6894-1>
- Lal R, Bruce JP. The potential of world cropland soils to sequester C and mitigate the greenhouse effect. *Environ Sci Policy.* 1999;2:177–85. [https://doi.org/10.1016/S1462-9011\(99\)00012-X](https://doi.org/10.1016/S1462-9011(99)00012-X)
- Law QD, Bigelow CA, Patton AJ. Selecting turfgrasses and mowing practices that reduce mowing requirements. *Crop Sci.* 2016;56: 3318–27. <https://doi.org/10.2135/cropsci2015.09.0595>
- Leino L, Tall T, Helander M, Saloniemi I, Saikkonen K, Ruuskanen S, et al. Classification of the glyphosate target enzyme (5-enolpyruvylshikimate-3-phosphate synthase) for assessing sensitivity of organisms to the herbicide. *J Hazard Mater.* 2021;408:124556. <https://doi.org/10.1016/j.jhazmat.2020.124556>
- Maggi F, la Cecilia D, Tang FHM, McBratney A. The global environmental hazard of glyphosate use. *Sci Total Environ.* 2020;717:137167. <https://doi.org/10.1016/j.scitotenv.2020.137167>
- Mchunu C, Chaplot V. Land degradation impact on soil carbon losses through water erosion and CO₂ emissions. *Geoderma.* 2012;177–178:72–9. <https://doi.org/10.1016/j.geoderma.2012.01.038>
- Michalk DL, Millar GD, Badgery WB, Broadfoot KM, 2013. Revitalising Grasslands to sustain our communities: Proceedings, 22nd International Grassland Congress, 15–19 September, 2013, Sydney, Australia. Revitalising Grasslands to sustain our communities: Proceedings, 22nd International Grassland Congress, 15–19 September, 2013, Sydney, Australia.
- Mohamed M, Aliyat FZ, Ben Messaoud B, Simone C, Marina M, Filippo G, et al. Effects of pesticides use (glyphosate & paraquat) on biological nitrogen fixation. *Water Air Soil Pollut.* 2021;232:419. <https://doi.org/10.1007/s11270-021-05367-x>
- Muñoz-Rojas M. Soil quality indicators: critical tools in ecosystem restoration. *Curr Opin Environ Sci Health.* 2018;5:47–52. <https://doi.org/10.1016/j.coesh.2018.04.007>
- Muola A, Fuchs B, Laihonon M, Rainio K, Heikkonen L, Ruuskanen S, et al. Risk in the circular food economy: glyphosate-based herbicide residues in manure fertilizers decrease crop yield. *Sci Total Environ.* 2021;750:141422. <https://doi.org/10.1016/j.scitotenv.2020.141422>
- O'Mara FP. The role of grasslands in food security and climate change. *Ann Botany.* 2012;110:1263–70. <https://doi.org/10.1093/aob/mcs209>
- Paul EA, Paustian KH, Elliott ET, Cole CV. Soil Organic matter in temperate agroecosystems: long-term experiments in North America. Boca Raton: CRC Press; 2019.
- Poeplau C, Zopf D, Greiner B, Geerts R, Korvaar H, Thumm U, et al. Why does mineral fertilization increase soil carbon stocks in temperate grasslands? *Agric Ecosyst Environ.* 2018;265:144–55. <https://doi.org/10.1016/j.agee.2018.06.003>
- Porra RJ, Thompson WA, Kriedemann PE. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochim Biophys Acta Bioenerg.* 1989;975:384–94. [https://doi.org/10.1016/S0005-2728\(89\)80347-0](https://doi.org/10.1016/S0005-2728(89)80347-0)
- van der Putten WH, Peters BAM. How soil-borne pathogens may affect plant competition. *Ecology.* 1997;78:1785–95. [https://doi.org/10.1890/0012-9658\(1997\)078\[1785:HSBPM\]2.0.CO;2](https://doi.org/10.1890/0012-9658(1997)078[1785:HSBPM]2.0.CO;2)

- Ramankutty N, Evan AT, Monfreda C, Foley JA. Farming the planet: 1. Geographic distribution of global agricultural lands in the year 2000. *Global Biogeochem Cycles*. 2008;22. <https://doi.org/10.1029/2007GB002952>
- Ramula S, Mathew SA, Kalske A, Nissinen R, Saikkonen K, Helander M. Glyphosate residues alter the microbiota of a perennial weed with a minimal indirect impact on plant performance. *Plant Soil*. 2021;472:161–74. <https://doi.org/10.1007/s11104-021-05196-1>
- Riedo J, Wettstein FE, Rösch A, Herzog C, Banerjee S, Büchi L, et al. Widespread occurrence of pesticides in organically managed agricultural soils—the ghost of a conventional agricultural past? *Environ Sci Technol*. 2021;55:2919–28. <https://doi.org/10.1021/acs.est.0c06405>
- Ruuskanen S, Fuchs B, Nissinen R, Puigbò P, Rainio M, Saikkonen K, et al. Ecosystem consequences of herbicides: the role of microbiome. *Trends Ecol Evolut*. 2023. <https://doi.org/10.1016/j.tree.2022.09.009>
- Saari S, Helander M, Lehtonen P, Wallius E, Saikkonen K. Fungal endophytes reduce regrowth and affect competitiveness of meadow fescue in early succession of pastures. *Grass Forage Sci*. 2010;65:287–95. <https://doi.org/10.1111/j.1365-2494.2010.00746.x>
- Saikkonen K, Gundel PE, Helander M. Chemical ecology mediated by fungal endophytes in grasses. *J Chem Ecol*. 2013;39:962–8. <https://doi.org/10.1007/s10886-013-0310-3>
- Saikkonen K, Lehtonen P, Helander M, Koricheva J, Faeth SH. Model systems in ecology: dissecting the endophyte–grass literature. *Trends Plant Sci*. 2006;11:428–33. <https://doi.org/10.1016/j.tplants.2006.07.001>
- Saikkonen K, Saari S, Helander M. Defensive mutualism between plants and endophytic fungi? *Fungal Divers*. 2010;41:101–13. <https://doi.org/10.1007/s13225-010-0023-7>
- Saikkonen K. Evolution of endophyte–plant symbioses. *Trends Plant Sci*. 2004;9:275–80. <https://doi.org/10.1016/j.tplants.2004.04.005>
- Skinner C, Gattinger A, Krauss M, Krause H-M, Mayer J, van der Heijden MGA, et al. The impact of long-term organic farming on soil-derived greenhouse gas emissions. *Sci Rep*. 2019;9:1702. <https://doi.org/10.1038/s41598-018-38207-w>
- Tsiafouli MA, Thébault E, Sgardelis SP, de Ruiter PC, van der Putten WH, Birkhofer K, et al. Intensive agriculture reduces soil biodiversity across Europe. *Global Change Biol*. 2015;21:973–85. <https://doi.org/10.1111/gcb.12752>
- Wagg C, Schlaeppi K, Banerjee S, Kuramae EE, van der Heijden MGA. Fungal-bacterial diversity and microbiome complexity predict ecosystem functioning. *Nat Commun*. 2019;10:4841. <https://doi.org/10.1038/s41467-019-12798-y>
- Wall DH, Nielsen UN, Six J. Soil biodiversity and human health. *Nature*. 2015;528:69–76. <https://doi.org/10.1038/nature15744>
- Ward SE, Smart SM, Quirk H, Tallowin JRB, Mortimer SR, Shiel RS, et al. Legacy effects of grassland management on soil carbon to depth. *Global Change Biol*. 2016;22:2929–38. <https://doi.org/10.1111/gcb.13246>
- Wilkes TI, Warner DJ, Davies KG, Edmonds-Brown V. Tillage, glyphosate and beneficial arbuscular mycorrhizal fungi: optimising crop management for plant–fungal symbiosis. *Agriculture*. 2020;10:520. <https://doi.org/10.3390/agriculture10110520>
- Wittwer RA, Bender SF, Hartman K, Hydbom S, Lima RAA, Loaiza V, et al. Organic and conservation agriculture promote ecosystem multifunctionality. *Sci Adv*. 2021;7:eabg6995. <https://doi.org/10.1126/sciadv.abg6995>
- Zobiolo LHS, Kremer RJ, de Oliveira RS Jr., Constantin J. Glyphosate effects on photosynthesis, nutrient accumulation, and nodulation in glyphosate-resistant soybean. *J Plant Nutr Soil Sci*. 2012;175:319–30. <https://doi.org/10.1002/jpln.201000434>

How to cite this article: Keronen S, Helander M, Saikkonen K, Fuchs B. Management practice and soil properties affect plant productivity and root biomass in endophyte-symbiotic and endophyte-free meadow fescue grasses. *J Sustain Agric Environ*. 2022;1–10. <https://doi.org/10.1002/sae2.12035>