UNIVERSITY OF TURKU
Faculty of Science

Effects of cutting height, fungal symbiont and soil properties on meadow fescue’s growth

Sanna-Maria Keronen

Ecology and Evolutionary Biology
Master’s thesis
Credits: 30 ECTs

Supervisors:
Benjamin Fuchs
Marjo Helander
Kari Saikkonen

5.5.2022
Turku

Master's thesis

Subject: Ecology and Evolutionary Biology
Author: Sanna-Maria Keronen
Title: Effects of cutting height, fungal symbiont and soil properties on meadow fescue's growth
Supervisors: Dr. Benjamin Fuchs, Dr. Marjo Helander, Dr. Kari Saikkonen
Number of pages: 43 pages
Date: 5.5.2022

Abstract

Grasses have a beneficial effect on the soil organic matter content, and hence carbon sequestration of agricultural soils throughout the world. Because grasslands cover one-third of the land on Earth and meadows and pastures approximately 70% of the world's agricultural area, grasses serve as a powerful carbon sink. Meadow fescue is an important perennial pasture and forage grass in Scandinavia. It has deep roots, rapid growth in spring, high after growth ability, low straw formation and excellent winter hardiness. It has excellent forage quality and amount of yield and superior fiber digestibility. Meadow fescue cultivars are often symbiotic with seed transmitted systemic fungal endophytes (*Epichloë uncinata*) offering various benefits for their hosts, for example increased growth and reproduction, and resistance to herbivores, pathogens and abiotic environmental stresses, and systemic fungal endophytes thereby enhance the competitive abilities of endophyte-infected plants. In my master's thesis I studied the effects of cutting height, residues of glyphosate based herbicides (GHBs) in soil, sterilized soil and fungal endophyte *Epichloë uncinata* to aboveground biomass production, root biomass and chlorophyll content in a greenhouse experiment. To test the importance of cutting height to grass performance, I assigned grasses to three different cutting treatments: uncut, cut to the height of 5 cm and cut to the height of 15 cm. Half of the plants were symbiotic to the fungal endophyte, the other half were endophyte-free. Plants were assigned to three different soil groups: control, GBH treated and sterilized soil. Cutting height significantly affected the total aboveground plant biomass, root biomass and chlorophyll content. Uncut meadow fescues produced the largest total aboveground biomass and root biomass which were significantly higher compared to total aboveground biomass and root biomass from grasses cut to 15 cm or 5 cm. The grasses cut to the height of 5 cm had the smallest total aboveground biomass and root biomass, which were further significantly smaller compared to grasses cut to 15 cm. Endophyte did not affect the total aboveground biomass, root biomass or the chlorophyll content of the plants. Meadow fescues produced higher amount of total aboveground biomass when growing in a sterilized soil compared to control or GBH treated soil. Root biomass was not significantly affected. Glyphosate residues in the soil decreased the amounts of total aboveground and root biomass of grasses. Chlorophyll content was highest in plants growing in sterilized soil. I conclude that to improve the total aboveground plant biomass and root biomass, the use of GBHs should be avoided and rotational grazing, in which animals are moved between pastures before they have been eating the grass too low, should be preferred. This will enable farmers to collect more harvest and sequester more carbon at the same time.

Keywords: *Schedonorus pratensis, Epichloë uncinata,* systemic fungal endophyte, glyphosate, soil sterilization, cutting treatment, grazing, silage

Contents

1 Introduction

1.1 Carbon and soil

Soil carbon sequestration is a key mitigation strategy for rising carbon dioxide concentration in the atmosphere, and important in improving the fertility and quality of soil. Carbon stored in soils worldwide exceeds the amount of carbon stored in phytomass and the atmosphere (Jobbágy & Jackson 2000). There is no consensus of the size of global soil organic carbon stocks, but most studies report a global soil organic carbon estimate to be roughly 1500 petagrams of carbon to a depth of 1 meter and 2400 Pg of carbon to 2 meter depth (Pg C: 10_{15} g or billion tons of carbon) (Scharlemann et al. 2014; Paustian et al. 2019). Globally agricultural land covers approximately 5 billion hectares, or 38 percent of the global land surface. About 33 percent of this is used as cropland and 67 percent consist of pastures for grazing livestock (FAO 2020). At the moment agricultural lands have been seriously degraded by widespread, unsustainable management. Soil quality is directly linked to food production, food security and environmental quality through its effects of energy use in food production, greenhouse gas emissions and water quality. Soil quality describes the capacity of soil to function as a provider of key ecosystem services, such as decomposing organic matter, supplying and cycling of nutrients for optimum plant growth, filtering water passing through soil to support clean groundwater, receiving rainfall and storing water for root utilization, and storing organic carbon for nutrient retention and mitigating greenhouse gas emissions (Franzluebbers 2012; O'Mara 2012).

Soils growing perennial grasses have high organic matter content and they can contribute to an agricultural future with high soil quality, and therefore can mitigate greenhouse gas emissions through soil carbon sequestration and improve a multitude of other ecosystem responses, including controlling water quality, improving water and nutrient cycling, and supporting biological diversity. Agricultural soils would benefit from the reintroduction of perennial grasses to regain soil organic matter and strengthen their capacity for long-term productivity and environmental resiliency (Franzluebbers 2012). Crop production results in a loss of soil organic carbon due to decreasing carbon inputs in the soils and by causing soil erosion (Bakker et al. 2004; Kirkels et al. 2014; Doetterl et al. 2016). Soil erosion affects vegetation growth and biomass production by changing physical and chemical properties of soil related to soil fertility, such as water holding capacity, nutrient status and soil depth (Bakker et al. 2004). Intensification of agricultural practices, like ploughing and the application of agrochemicals and artificial fertilizer, are linked to a reduced soil biodiversity (Tsiafouli et al. 2014).

Land use, for example whether the area is primary forest, plantation, pasture or growing crop, affects to the soil carbon levels. Soil carbon stocks increase when area is converted from crop to pasture

(+19%), from forest to pasture (+8%), from crop to plantation (+18%) and from crop to secondary forest (+53%) (Guo & Gifford 2002). Soil carbon declines when area is converted from pasture to plantation (-10%), from pasture to crop (-59%), from forest to crop (-42%) and from forest to plantation (-13%) (Guo & Gifford 2002). Pasture grasses maintain a cover of vegetation on the soil, add organic matter on both above- and belowground, and reduce soil temperatures (Brown & Lugo 1990). The annual carbon input to soil from crop residues originates from straw, stubble and surface debris, and root biomass left in the soil at harvest, root turnover, exudates and secretions (Bolinder et al. 2002). Perennial grasses have a beneficial effect on the soil organic matter content, and hence carbon sequestration of agricultural soils throughout the world. This is mostly because of the root biomass production of perennial forage crops and the absence or reduction of tillage compared to annual crops (Paustian et al. 1997). Well-managed grasslands can maintain and accumulate soil carbon and hence contribute to climate change mitigation (Leifeld & Fuhrer 2010; Soussana et al. 2014; LaCanne & Lundgren 2018; Poeplau et al. 2018).

The soil is where most terrestrial plants start their growth, and home to a diverse community of microbes and animals. The soil is the source of most beneficial microbes that colonize the rhizosphere, and that are key players in plant immunity and overall plant performance (Bulgarelli et al. 2013). Soil microbes provide plants with key functions, such as enhanced growth via improved nutrition and suppression of soil pathogens (Pieterse et al. 2016; Raaijmakers & Mazzola 2016). An important soil service is also the protection of above-ground plant tissues against pests and diseases (Bardgett & Wardle 2010; Mariotte et al. 2018). Plants provide the organic carbon for the decomposers and the resources for root-associated organisms, for example root herbivores, pathogens, and symbiotic mutualists (Wardle et al. 2004). Decomposers break down dead plant material and thereby regulate plant growth and community composition by determining the supply of available soil nutrients. Root-associated organisms and their consumers influence the quality, direction, and flow of nutrients and energy between plants and decomposers (Wardle et al. 2004). The complexity of soil foodweb is essential to maintain high rates of ecosystem function. Activities that contribute belowground biodiversity losses, such as loss of taxa and trophic levels, cause a reduction in foodweb complexity and the capacity of soils to perform ecosystem functions (Wall et al. 2015).

1.2 Removing biomass from perennial grass fields

Grasses can tolerate repeating disturbance well, because by their evolution they have adapted to it. For example pastures are continually grazed, and lawns and fields are mowed. In addition grass breeding has increased for example to the forage yields and regrowth, and improved resistance against pests and pathogens (Saari et al. 2010). In addition to the fast regrowth, grasses can tolerate grazing also because of their underground storage organs, basal meristems and tillering capacity (Huitu et al. 2014).

Herbivores alter carbon and nitrogen inputs to the soil by changing the quality and quantity of organic inputs (for example herbivore dung and plant litter), by decreasing biological nitrogen fixation through the consumption of legumes, and through changes in soil conditions, like temperature and moisture (Bardgett & Wardle 2003; Pineiro et al. 2010), which in turn have an impact in soil microbial communities and activity (Bardgett & Wardle 2010). Greater microbial activity increases litter decomposition rates and carbon respiration and also carbon transfer into slow-cycling forms of carbon, for example microbial necromass (Lange et al. 2015; Sokol & Bradford 2019), which further increases the potential for carbon sequestration under grazing. Under eutrophied conditions herbivores promote soil carbon and nitrogen storage in grasslands (Sitters et al. 2020).

The negative effect of defoliation on growth rate or final biomass is typically less than proportional to the removal of live biomass. Sometimes it can even be positive (McNaughton et al. 1983). When defoliated plants can partially or fully compensate for the removal of biomass, the response is known as compensatory regrowth (Ferraro & Oesterheld 2002). The magnitude how much grasses can compensate defoliation, are dependent on evolutionary mechanisms, for example coevolutionary history with big vertebrate grazers (Vail 1992), nutrient levels (Georgiadis et al. 1989; Alward & Joern 1993; Ferraro & Oesterheld 2002), carbon allocation (Briske et al. 1996), recovery conditions (Oesterheld & McNaughton 1988; Ferraro & Oesterheld 2002) and light environment (McNaughton 1992). In addition defoliation can affect to the root growth and belowground carbohydrate reserves, decreasing root biomass (Holland et al. 1996; Thornton & Millard 1996) and belowground growth rate (Oesterheld 1992).

A species of animal, frequency and severity of grazing, method of prehension, treading, excreta deposited on pastures, and saliva deposited on plants during grazing influence to the plants that are grazed. These animal factors can cause substantial changes in the persistence, productivity, and botanical composition of the sward and the regrowth rate of plants following grazing. The extent of leaf-tissue removal, the accumulation of dead material, canopy structure in relation to light interception, the microenvironment within the canopy, species composition of the sward and the general physiological well-being of plants will be altered by the intensity and frequency of grazing (Matches 1992). Saliva of grazing animals might have an important role for increasing grass growth (Reardon et al. 1974; Gullap et al. 2011). Grazing intensity of pastures should be regulated to maintain adequate leaf area for maximum plant growth rates throughout the grazing season (Matches 1992). Meadow fescue has been shown to have a bigger regrowth if it is cut from the height of 9 cm instead of 3 cm (Virkajärvi 2003).

1.3 Meadow fescue (*Schedonorus pratensis*)

Meadow fescue [*Schedonorus pratensis* (Huds.) P. Beauv syn. *Festuca pratensis* (Huds.) and *Lolium pratense* (Huds.)] is an important forage grass in temperate and cold climates. It is perennial and has height of 40-100 cm. It grows as a tuft formation, has rapid growth in spring, high after growth ability and low straw formation. Meadow fescue has an excellent winter hardiness and forage quality. Meadow fescue tends to be outcompeted from multispecies grasslands, because its competitive ability is relatively low. Meadow fescue can cross-breed with tall fescue, perennial ryegrass (*Lolium perenne*) and Italian ryegrass (*Lolium multiflorum*) (Fjellheim & Rognli 2005; Brink et al. 2010; Saari et al. 2010; Rao & Rognli 2014; Rikkinen 2014). Meadow fescue is a viable alternative for example to tall fescue and orchardgrass in managed intensive rotational grazing systems because of its comparable yield and superior fiber digestibility. Meadow fescue has higher nutritional quality than for example tall fescue (*Schedonorus arundinacea*) or orchardgrass (*Dactylis glomerata*). (Brink et al. 2010). Both cattle and horses prefer meadow fescue in their diet (Allen et al. 2013).

1.4 Systemic fungal endophyte *Epichloë uncinata*

Meadow fescue's fungal endophyte *Epichloë uncinata* [(W. Gams, Petrini & D. Schmidt) Leuchtm. & Schardl syn. *Neotyphodium uncinatum*] subsists entirely on the host's resources, and the fitness of an endophytic symbiont depends on the fitness of the host plant. The host, meadow fescue, receives various benefits, for example increased growth and reproduction, and resistance to herbivores, pathogens and abiotic environmental stresses, which thereby enhance the competitive abilities of endophyte-infected plants (Saikkonen et al. 2004; Lehtonen et al. 2005). *Epichloë uncinata* endophytes in meadow fescue plants are producing loline alkaloids which are known to have detrimental effects on invertebrates (Hume et al. 2009; Jensen et al. 2009; Popay et al. 2007; Popay et al. 2009; Patchett et al. 2011), but have not known effect on grazing mammals (Schardl et al. 2007). However, female voles have been shown to suffer from weight lost because of loline alkaloids (Huitu et al. 2014). The genotype of meadow fescue affects the amount of alkaloids and fungal mass in the host (Cagnano et al. 2020). There are several combinations and concentrations of alkaloids, and their presence is dependent on for example environmental conditions (Spiering et al. 2005).

Systemic fungal endophytes cannot exist without its grass host (except as a cultured mycelia on artificial media), because they need the host grass for supply for nutrients and water and dissemination through seeds of the host plant to the next plant generation. Systemic *Epichloë* endophytes grow internally and intercellularly throughout the above-ground tissues of the host plant and into the developing inflorescences and seeds (Saikkonen et al. 2004; Lehtonen et al. 2005). The coiled hyphae of fungal endophytes do not penetrate the plant cells (Cheplick & Faeth 2009).

Epichloë uncinata reproduces asexually via the plant seeds. The fitness of the symbiotic endophyte is totally dependent the fitness of the grass host. *Epichloë uncinata* provides benefits to its host, such as competitive advantage against other plants, and higher shoot and root dry weight comparing to endophyte-free meadow fescues. Extensive root system is a vital characteristic for drought avoidance, and most likely increases persistence of endophyte-symbiotic meadow fescue under drought and when competing with other species (Malinowski et al. 1997; Malinowski & Belesky 2000; Saari et al. 2010). The symbiosis between *Epichloë uncinata* and meadow fescue can range from antagonistic to mutualistic depending on the genetic match of the fungal endophyte and the host grass, and also environmental conditions (Ahlholm et al. 2002; Saikkonen et al. 2004; Saikkonen et al. 2010). Nutrients are vital for the mutualism between meadow fescue and *Epichloë uncinata.* When resources are limited, endophyte-symbiotic meadow fescue produces fewer tillers and seeds, and has lower total biomass and lower root mass compared to endophyte-free meadow fescue (Ahlholm et al. 2002). The positive effect from the endophyte to the grass host is strongest in the environments where nutrients are not a limiting factor, e.g. often in the agroenvironments (Saikkonen et al. 2006; Cheplick & Faeth 2009; Saikkonen et al. 2013). Endophyte infection affects host's morphology, chemistry and physiology. It alters for example leaf rolling, photosynthesis, nutrient uptake, water-use efficiency, hormonal changes and root morphology (Malinowski & Belesky 2000; Morse et al. 2007; Cheplick & Faeth 2009). Study with perennial ryegrass has shown, that endophyte infection interacts with levels of lipids, carbon and nitrogen, amino acids, magnesium, organic acid, chlorogenic acid, nitrogen availability to alter nitrate and water soluble carbohydrates (Rasmussen et al. 2008).

Endophyte-symbiotic meadow fescues may survive better in salinity-stress environments compared to endophyte-free meadow fescues (Sabzalian & Mirlohi 2010). Fungal endophyte helps also to prevent virus infections in its host grass, probably by deterring of herbivorous insects, which carry plant viruses (Lehtonen et al. 2006). It has been shown that endophyte infection increases root hair length and decreases root diameter in tall fescue grasses. That may increase root surface area for water and mineral acquisition (Malinowski & Belesky 1999).

Endophyte-symbiotic meadow fescue has more panicles, greater reproductive shoot mass and higher total seed mass than endophyte-free meadow fescue, but this is context dependent. Plant genotype (e.g.cultivar) and environmental conditions (e.g. nutrients) are affecting to outcome of the symbiosis (Wäli et al. 2008). The total biomass of endophyte-symbiotic meadow fescue monocultures was 89% higher than endophyte-free meadow fescue monocultures in high nutrient conditions (Dirihan *et al.* 2014). The effect of the endophyte on the annual herbage yield of the meadow fescue monocultures varies between the cultivars (Takai et al. 2010). On the other hand, in pastures growing multiple grass species, when meadow fescues have been young, their fungal endophytes have not increased their ability to compete with other grass species and against herbivores, but in older pastures and ungrazed fields fungal endophytes have increased grasses ability to compete with other grass species and against herbivores (Saari et al. 2010; see also Fuchs et al. 2016; Fuchs et al. 2017; Hewitt et al. 2020.)

1.5 Glyphosate based herbicides

Today glyphosate based herbicides (GBHs) are globally the most used herbicides (Luonnonvarakeskus 2019; Uusi-Kämppä 2019). Glyphosate, N-(phosphonomethyl) glycine, has been in use extensively the past 40 years. It was introduced for weed control in agricultural production fields in 1974 (van Bruggen et al. 2018). Glyphosate is a non-selective, systemic, post-emergence herbicide, and it acts as an inhibitor of the enzyme, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), in the shikimate pathway (Duke & Powles 2008). Shikimate pathway is a metabolic pathway plants use for the biosynthesis of aromatic amino acids (Gill et al. 2017). Besides plants shikimate pathway is found in many microbes (Leino et al. 2020). Scarcity of the enzyme leads to the deficiency of aromatic amino acids, which in turn affects various metabolic functions of the plant and causes the plant to wither (Tu et al. 2001). Glyphosate depletes pools of compounds needed for carbon fixation, which causes a general disruption of the organism's metabolism (Duke & Powles 2008). In addition usage of glyphosate can affect negatively to chlorophyll levels of plants, even when the plants are glyphosate-resistant (Zobiole et al. 2012). In 2018 1261 000 kilograms of glyphosate based herbicides were applied on Finnish fields. GBHs are frequently used for weed control before establishing new perennial grass fields in Finland. GBHs can be sprayed after the harvest in the autumn or before sowing in the spring (Luonnonvarakeskus 2019; Uusi-Kämppä 2019).

Intensive glyphosate use has led to the selection of glyphosate-resistant weeds and microorganisms (Powles & Preston 2006). There are also reported health effects in animals associated with chronic, ultra-low doses related to accumulation of glyphosate and its breakdown product aminomethyl phosphonic acid (AMPA) in the environment (Shushkova et al. 2009; van Bruggen et al. 2018). Glyphosate's half-life in soil ranges from 2 to 215 days, and an aquatic half-life ranges from 2 to 91 days (Giesy et al. 2000; Grunewald et al. 2001; Vera et al. 2010). Glyphosate degrades primarily by microbial processes to AMPA. AMPA, like glyphosate, is very water soluble, and it degrades slower than glyphosate (Grunewald et al. 2001). AMPA's half-life in soil ranges from 60 to 240 days, and an aquatic half-life is similar than glyphosate's aquatic half-life (Giesy et al. 2000; Bergstrom et al. 2011). AMPA degrades to inorganic phosphate, ammonium and CO^2 (Borggaard & Gimsing 2008). AMPA's degradation process can result a substantial increase of total phosphorous in aquatic systems (Vera et al. 2010). Glyphosate adsorbs to clay and organic matter, which slows its degradation by soil microorganisms, and leads to accumulation in soils over time (Banks et al. 2014; Sviridov et al. 2015; Okada et al. 2016).

Glyphosate and AMPA inhibit antioxidant enzyme activities and induce the accumulation of reactive oxygen species (ROS), which result to physiological dysfunction and cell damage (Gomes et al. 2016). Glyphosate and AMPA decrease photosynthesis. Glyphosate increases chlorophyll degradation, and AMPA disturbs biosynthesis of chlorophyll (Gomes et al. 2016). Even sublethal glyphosate concentrations in plants, for example from residues in soil or water, decrease plant resistance to pathogens. Infection by *Fusarium* species is often more severe in fields treated by glyphosate before planting compared with untreated control fields (Kremer et al. 2005; St. Laurent et al. 2008; Kremer & Means 2009; van Bruggen et al. 2015). Besides reduced plant resistance, indirect effects of glyphosate and AMPA to the health of plants are possible through changes in the endophytic and rhizosphere microbiome (Kremer et al. 2005; Kuklinsky-Sobral et al. 2005; Berg et al. 2014; van Bruggen et al. 2016), because many microbes are sensitive to glyphosate (Leino et al. 2020). Soil microbes play diverse and critical role in soil systems and plants performance (Aislabie & Deslippe 2013; Wall et al. 2015; Wagg et al. 2019).

Surfactants and other adjuvants are added to commercial glyphosate formulations to enhance their efficacy. Commercial GBH formulations can be more toxic than pure glyphosate due to the toxicity and action of the surfactants and other adjuvants used (Giesy et al. 2000; Edginton et al. 2004; Bringolf et al. 2007; Mesnage et al. 2012; Moore et al. 2012; Sihtmäe et al. 2013). For example the Roundup® formulation is more toxic than glyphosate or AMPA for all taxa tested (Giesy et al. 2000).

1.6 The aims and hypothesis of this study

Grasses have evolved to tolerate high pressure from vertebrate grazers. In agricultural fields perennial grasses are used in pastures for animals grazing or cut for animals as a form of hay or silage. I chose meadow fescue for my study species, because it is commonly used in Finnish pastures and silage fields, and has not been studied as often as for example tall fescue or perennial ryegrass. GBH are commonly used herbicides in Finland for example in autumn when finishing the pasture or silage field or crop field before sowing it again. However, the effects of glyphosate residues in the soil on the growth and biomass production of meadow fescue have not been studied before. To better understand whether the effect of GBH in soil on meadow fescue plants is connected to its antimicrobial effect on the soil biota, I added sterilized soil as a treatment, where the soil biota is destroyed.

Grass for silage or hay is cut usually low, ca. 5-8 cm height from the soil surface. In this study I decided to compare uncut meadow fescue biomass production to cutting heights 5 cm and 15 cm from the soil surface.

My study questions are:
1. Does the endophytic fungus affect biomass production of meadow fescue under cutting treatment?
2. Does cutting height affect the total green biomass production, root biomass and chlorophyll content of meadow fescue?
3. Does soil treatment with GBH and the presence of the endophytic fungus affect to plants' total green biomass production, root biomass and chlorophyll content?

My first hypothesis is that endophytic fungi have a positive effect for growth and recovery after the cutting treatment based on their mutualistic nature. In addition my hypothesis is that endophyte-symbiotic grasses will produce more chlorophyll than grasses that are endophyte-free.

My second hypothesis is that cutting height affects the growth, green biomass production and root biomass production. I predict that cutting at 5 cm height will decrease grass after growth, the total aboveground and root biomass compared to grasses cut to a height of 15 cm. In addition I predict that cutting height and the presence of endophytic fungus affect the chlorophyll content of the plants.

My third hypothesis is that GBH and sterilized soil treatments decrease the total aboveground biomass and root biomass. Furthermore, I predict that growing in GBH treated soil and sterilized soil decreases the chlorophyll content of the plants compared to the control plants.

2 Material and methods

2.1 Study design and treatments

Meadow fescue seeds used for the study were collected in the previous year (2020) from meadow fescue plants with known endophyte status, that grew on an experimental field at the Ruissalo Botanical Garden in Turku. Seeds were checked for the endophyte status before they were used. 3-5 seeds from each plant were placed over night into an Eppendorf tube containing 1 ml of liquid (made by 0.9 ml of water, 0.1 ml of ethanol and 0.025 g of Sodium hydroxide). Next day, seeds were rinsed with tap water, then slightly crushed and microscopically analyzed. In endophyte-symbiotic plants the endophytic fungal hyphae was detected between the embryonic cells (Figure 1).

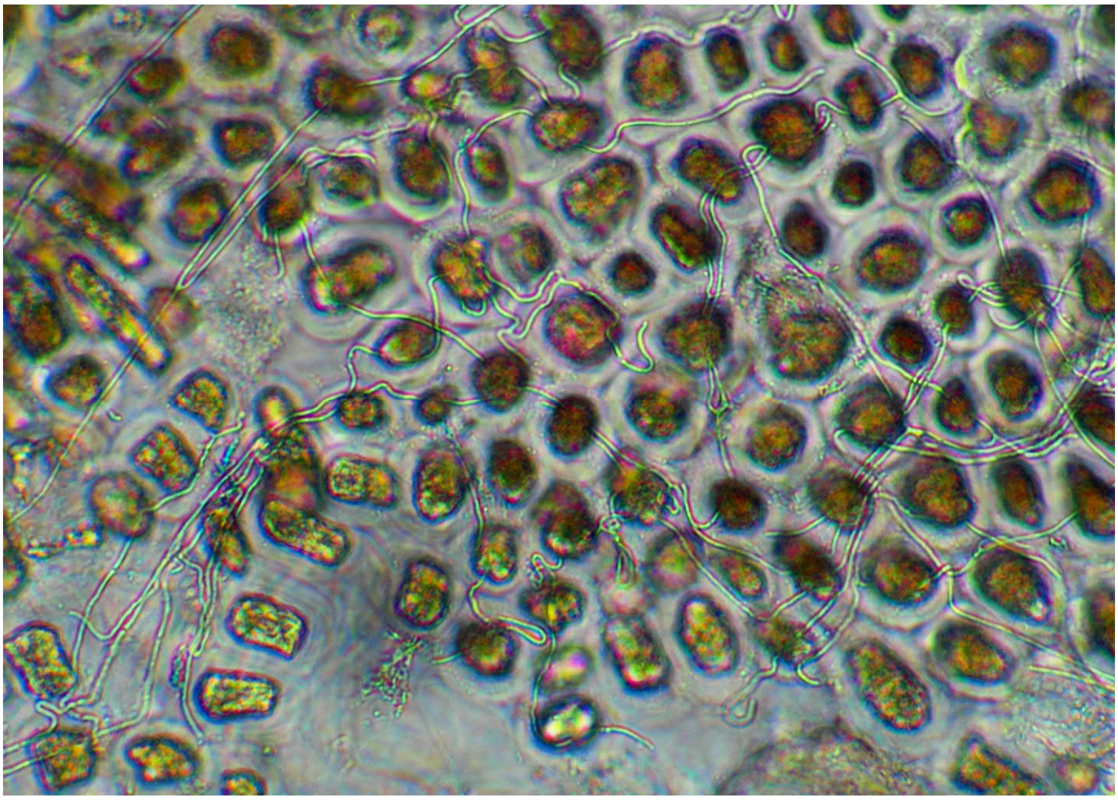

Figure 1. *Epichloë uncinata* hyphae between embryonic cells of the meadow fescue seed

Seeds from seven endophyte-symbiotic plants (E+) and seven endophyte-free (E-) plants were used in this study. Seeds from the seven E+ grasses were pooled, and similarly seeds from the seven E- plants were pooled before randomly selected sowing. Seeds were sown on 21.04.2021 to two planting trays that were filled with Kekkilä Garden Viherkasvimulta planting soil and watered. Each potting tray consisted of 260 units, and each unit was sown with 3-5 seeds. Units were thinned to one seedling on 04.05.2021. Both trays had 130 units with E+ seeds and 130 units with E- seeds that were randomly chosen from seed mixtures (Figure 2). The trays were covered with transparent plastic to keep the moisture in until the seeds germinated.

Figure 2. Meadow fescue seedlings growing on the trays

The soil used for planting the seedlings was collected from a long-term field experiment, which had been established in 2013 at the Botanical Garden of Ruissalo (Helander et al. 2019). The soil type of the field was medium clay. Before collecting the soil it was treated with lime (Tarhurin Puutarhakalkki, 10 kg/100 m^2, which had been spread in every spring on the field) and was tilled on 21.04.2021. The soil had been treated with a permitted dose of Roundup Gold® (450 g/l isopropylamine glyphosate salt, application rate: 6.4 l/ha) that had been applied twice per year (in the spring and in the autumn) since the year 2014. The control soil received the same amount sprayed tap water without Roundup® application. The last treatments before collecting the soil for this study were done on 29.04.2021.

The nutrient values of the soil were (based on an analysis made in 2016): pH 6.2 mg/l, phosphorus 4.2 mg/l, kalium 250 mg/l, calcium 1900 mg/l, magnesium 570 mg/l, sulfur 10.6 mg/l, zinc 2.74 mg/l, copper 7.5 mg/l, manganese 15 mg/l (Viljavuustutkimus 2016).

Soil for the pots from the experimental field was collected on 11.05.2021. Soil was collected from the area treated with GBH (glyphosate based herbicide, in this case Roundup®) and from the control (C) area. Part of the soil from the control area was heat-sterilized (S) in an autoclave (120 °C for 20 minutes). Soil was put in 1.5 liter pots which were put on three separate tables (one table for control pots, one for glyphosate pots and one for sterilized pots) to prevent contamination. Meadow fescue seedlings were transferred to the pots on the same day (11.05.2021). There were 60 pots of GBH soil, 60 pots of control (C) soil and 60 pots of sterilized (S) soil, each group contained 30 E+ seedlings and 30 E- seedlings. After the first clipping treatment the grasses were placed on the same table in a random order to guarantee similar environment (Figure 3).

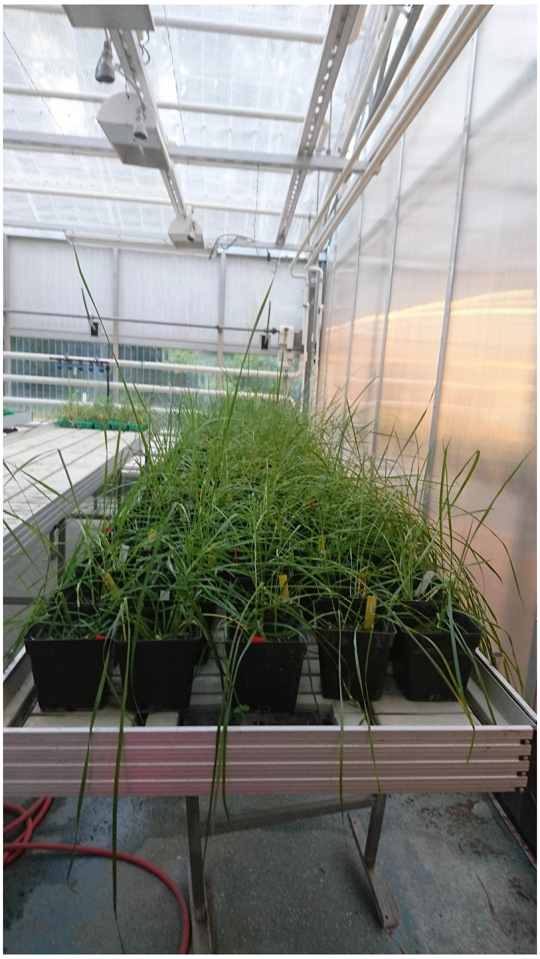

Figure 3. Meadow fescues from the different soil treatments, endophyte statuses and cutting treatments on the table in a random order

Plants were grown in a greenhouse with ambient light and temperature (20-26 °C). On 25.05.2021 the pots were weeded. High amount of weeds were growing in the GBH treated pots. Control pots had fewer weeds, and sterilized pots none. I also replaced dead plants (6 GBH and 1 control). Early stages of the study 5 grasses growing in GBH treated soil (8.3% of all grasses growing in GBH treated soil) and 1 grass growing in control soil died (1.7% of all grasses growing in control soil). Four of the dead plants died on the GBH group were from 5 cm cutting treatment (6.7% of all grasses growing in GBH treated soil), 1 was from the group that was cut from the height of 15 cm. The only dead plant in the control group was from 5 cm cutting treatment. Pots were weeded again on 06.06.2021, and GBH pots contained a high amount of weeds. Meadow fescues on GBH pots were smaller and thinner than meadow fescues on control and sterilized pots (personal observation). Unlike control and sterilized pots, GBH pots contained moss growth. Pots were regularly watered to avoid drought stress. Plants were grown in a greenhouse chamber until harvest of the aboveground biomass on 08.09.2021 (Figure 4).

Grasses were cut with normal scissors to the height of 5 cm and 15 cm (Figure 4). The cutting height was measured with a soft tape measurer. Grasses were cut on 18.06.2021, 09.07.2021 and 05.08.2021.

Figure 4. Ending the experiment by harvesting the green biomass

2.2 Biomass and chlorophyll content sampling

To study the effects of glyphosate residues in soil as well as soil sterilization treatment on plant performance the following plant parameters were recorded. The length of the longest leaf from meadow fescues was measured, the amount of tillers was counted and the SPAD value was measured on three occasions 17.06.2021, 08.07.2021 and 04.08.2021. The SPAD meter (Soil Plant Analysis Development) measures the difference between the transmittance of a red (650 nm) and an infrared (940 nm) light through the leaf, which enables the estimation of the amount of chlorophyll present on the leaf. The first assessments were carried out on sunny days; 17.06.2021 the air temperature outside was between 19°C and 22°C (in the greenhouse warmer) (Foreca 2021), 08.07.2021 the air temperature outside was between 21 °C and 25 °C (in the greenhouse warmer) (Foreca 2021), the third assessment on 04.08.2021 was carried out when it was a cloudy and partly rainy, when outside temperature was between 15 °C and 18 °C (Foreca 2021). The last day when SPAD values were taken (06.09.2021) the weather was mostly cloudy and temperature between +14 °C and +17 °C (Foreca 2021).

The SPAD (Minolta SPAD-502Plus meter) evaluations were taken on three randomly chosen leaves per plant, whereby the meter was placed randomly on leaf mesophyll tissue. Three SPAD readings were taken per plant and averaged to provide a single SPAD value. Diurnal chloroplast movements in response to light, affecting the degree of heterogeneity in the chlorophyll distribution, and therefore the SPAD values, should be minor, because all SPAD-measurements were conducted during 10:00-16:00 o'clock.

The fresh cut aboveground biomass parts from the cutting treatment plants (5 cm and 15 cm) were weighed on 18.06.2021, 09.07.2021 and 05.08.2021. After weighing the cut biomass was dried in oven at 65 °C for at least 48 hours and weighed again to get the dry weight. The scale used for weighing was same every time, Mettler Toledo AX204.

The experiment was concluded for 17 weeks after planting (06.-10.9.2021). The green biomass was cut and the fresh biomass was weighed, then leaves were dried and weighed again to gain the dry weight. Roots were carefully separated from the soil by rinsing them gently in water until they were clean. After washing, roots were dried with paper towels and weighed, then dried in the oven in the same manner as the leaves (at 65 °C for 48 hours in the drying oven) and weighed again.

I tested the effects of clipping and endophyte status, as well as their interaction term on total aboveground biomass, root biomass and chlorophyll content (SPAD value) via Analysis of Variance (ANOVA), before applying Tukey's Posthoc test comparing single treatments. Same response variables were analyzed with soil treatments and endophyte status as predictor variables. Total aboveground biomass was correlated with root biomass and a curve was fitted to the graph. Statistical analyses (ANOVA and correlation) were done in R.

3 Results

3.1 Effect of cutting height and endophyte status on plant parameters

Cutting height significantly affected the total aboveground plant biomass, root biomass and chlorophyll content of the meadow fescue plants (Table 1). Symbiotic endophytic fungi did not affect the biomass or chlorophyll content in any of the treatments.

Table 1. ANOVA summary table of plant responses to cutting treatment, endophyte treatment and their interaction term.

Response variable	Effector variable	Df	F	p
Total aboveground biomass	Cutting	2.168	75.74	**<0.0001**
	Endophyte	1.168	0.160	0.690
	Cutting*Endophyte	2.168	0.795	0.453
Root biomass	Cutting	2.168	110.1	**<0.0001**
	Endophyte	1.168	2.056	0.154
	Cutting*Endophyte	2.168	0.437	0.647
Chlorophyll (SPAD)	Cutting	2.168	38.35	**<0.0001**
	Endophyte	1.168	3.374	0.068
	Cutting*Endophyte	2.168	1.395	0.251

Uncut meadow fescues produced the largest total aboveground dry biomass, which was significantly higher compared to total aboveground dry biomass from grasses cut to 15 cm or 5 cm. The grasses cut to the height of 5 cm had the smallest total aboveground dry biomass, which was further significantly smaller compared to grasses cut to 15 cm (Figure 5). Similarly uncut meadow fescues produced a largest root dry biomass, which was significantly higher compared to root dry biomass from grasses cut to 15 cm or 5 cm (Table 2). The grasses cut from the height of 5 cm had the smallest root dry biomass, which was further significantly smaller compared to grasses cut to 15 cm (Figure 6). In addition uncut meadow fescues had the biggest chlorophyll content, which was significantly higher compared to the chlorophyll content from grasses cut to 5 cm. Uncut meadow fescues did not have significantly bigger chlorophyll content compared to the chlorophyll content from grasses cut to 15 cm (Table 2). The grasses cut from the height of 5 cm had the smallest chlorophyll content, which was further significantly smaller compared to grasses cut to 15 cm (Figure 7).

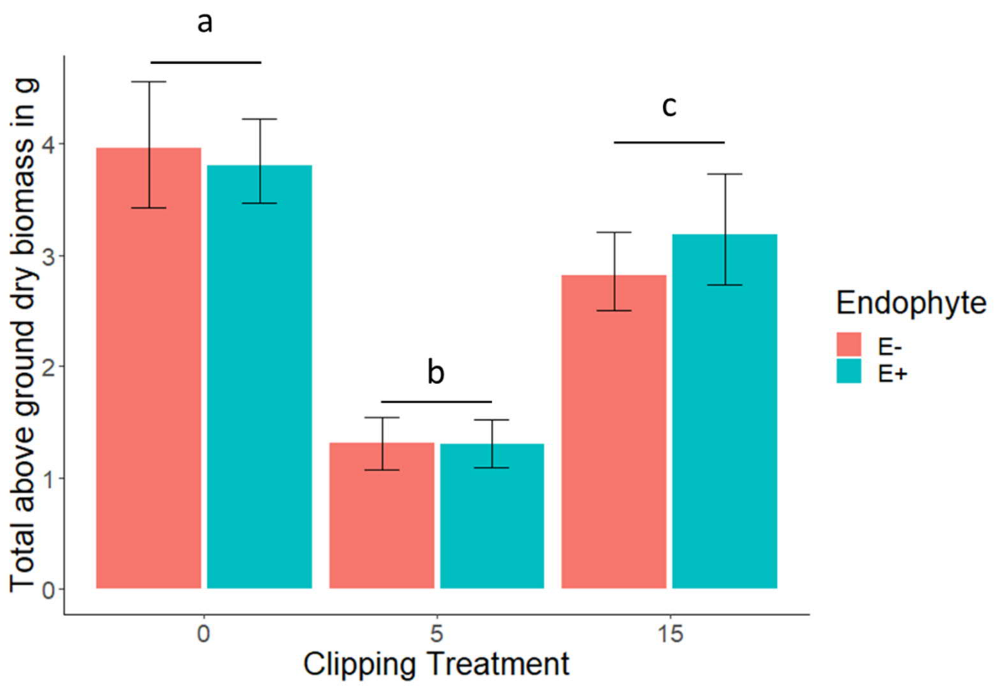

Figure 5. Total aboveground biomass was significantly different between the cutting heights (0= uncut, 5 cm, 15 cm), but was not affected by endophyte symbiosis. Letters indicate significant differences following Posthoc Tukey's tests (Table 2).

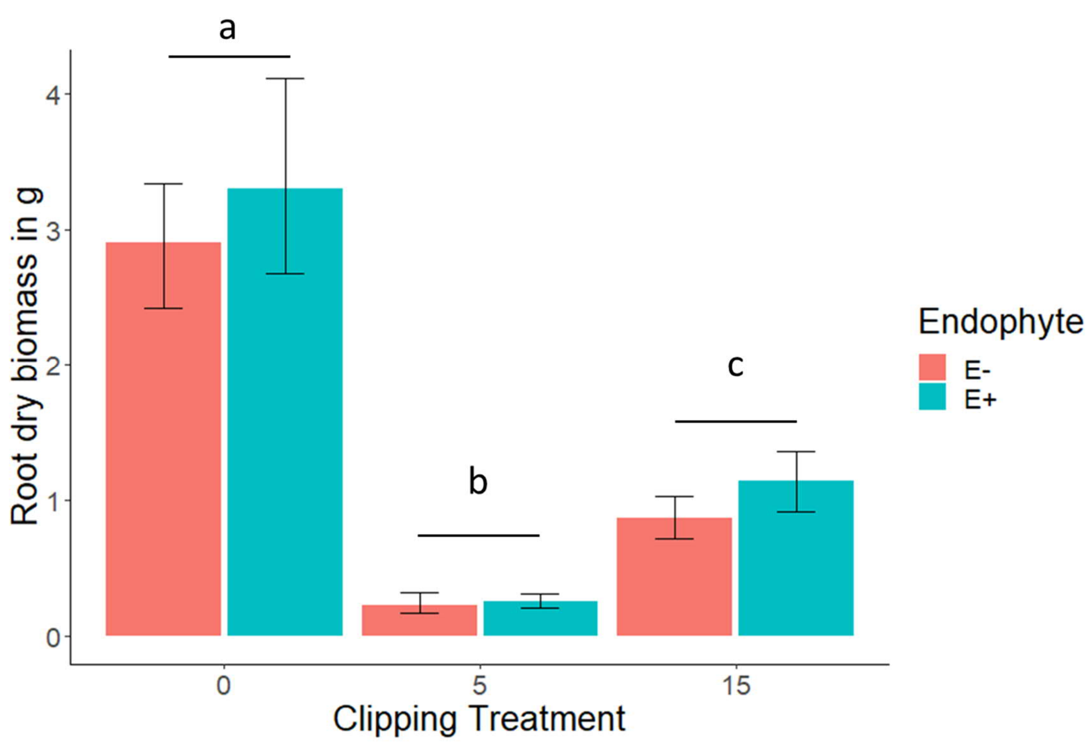

Figure 6. Root biomass was significantly different between the cutting heights (0= uncut, 5 cm, 15 cm), but was not affected by endophyte symbiosis. Letters indicate significant differences following Posthoc Tukey's tests (Table 2).

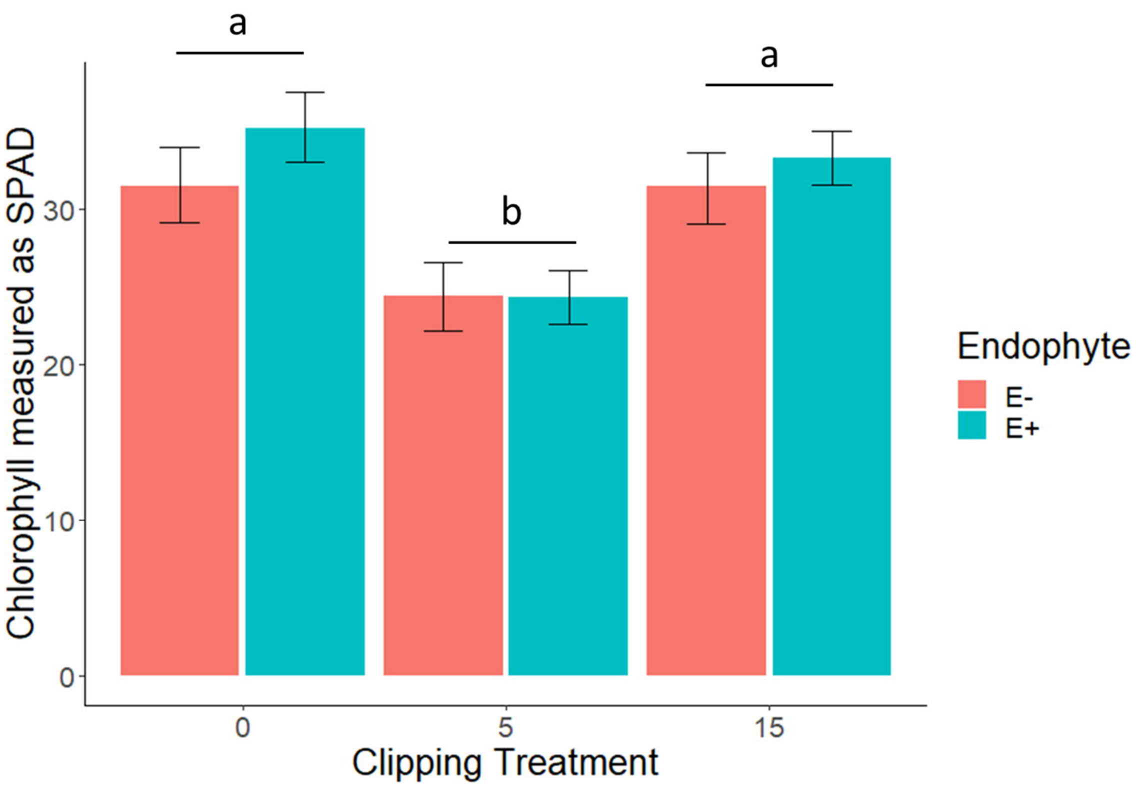

Figure 7. Chlorophyll content measured as SPAD was significantly different between the cutting heights (0= uncut, 5 cm, 15 cm), but was not affected by endophyte symbiosis. Letters indicate significant differences following Posthoc Tukey's tests (Table 2).

Table 2. Results from a Posthoc Tukey's test testing pairwise comparisons of plant parameters between the clipping treatments.

Total aboveground dry biomass		**T**	**p**
Uncut	Cut 5 cm	12.197	**<0.0001**
Uncut	Cut 15 cm	4.232	**0.0001**
Cut 5 cm	Cut 15 cm	8.008	**<0.0001**
Dry root biomass			
Uncut	Cut 5 cm	14.232	**<0.0001**
Uncut	Cut 15 cm	10.609	**<0.0001**
Cut 5 cm	Cut 15 cm	3.797	**0.0006**
Chlorophyll (SPAD)			
Uncut	Cut 5 cm	7.911	**<0.0001**
Uncut	Cut 15 cm	0.867	0.661
Cut 5 cm	Cut 15 cm	7.030	**<0.0001**

3.2 Effect of soil treatment and endophyte on plant parameters

Soil treatment significantly affected the total aboveground biomass (Table 3). Plants produced higher amount of total aboveground biomass when growing in a sterilized soil (S) compared to control (C) or glyphosate based herbicide treated (G) soil (Figure 8). Grasses growing in sterilized soil also had bigger leaf area compared to control or GBH group. Root biomass was not significantly affected even though there was a trend to higher biomass in the sterilized soil treatment (Table 3). Chlorophyll content measured as SPAD values were highest in plants growing in sterilized soil (Figure 9), but significantly different only in comparison to control plants (Table 4).

Table 3. ANOVA summary table of plant responses to soil treatment, endophyte treatment and their interaction term

Response variable	Effector variable	Df	F	p
Total aboveground biomass	Soil treatment	2.168	8.461	**0.0003**
	Endophyte	1.168	0.032	0.859
	Soil*Endophyte	2.168	0.182	0.834
Root biomass	Soil treatment	2.168	2.433	0.090
	Endophyte	1.168	0.654	0.420
	Soil*Endophyte	2.168	0.568	0.568
Chlorophyll (SPAD)	Soil treatment	2.168	4.392	**0.013**
	Endophyte	1.168	2.552	0.859
	Soil*Endophyte	2.168	0.226	0.834

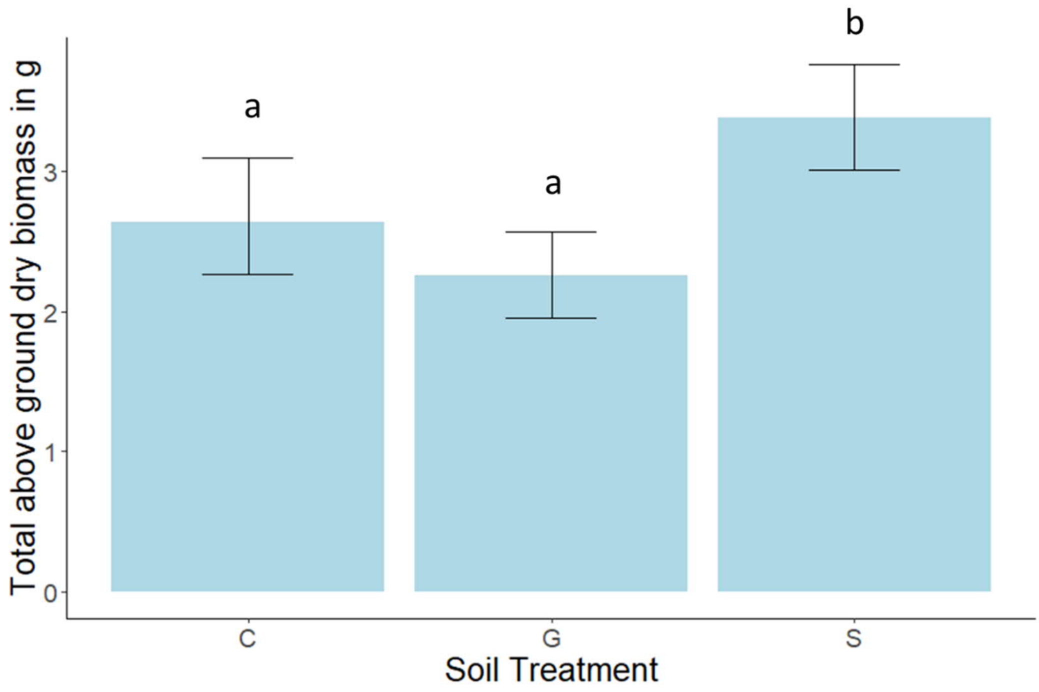

Figure 8. Total aboveground biomass was significantly different between soil treatments (C= control, G= glyphosate based herbicide, S= sterilized), but was not affected by endophyte symbiosis. Different letters indicate significant differences following Posthoc Tukey's tests (Table 4).

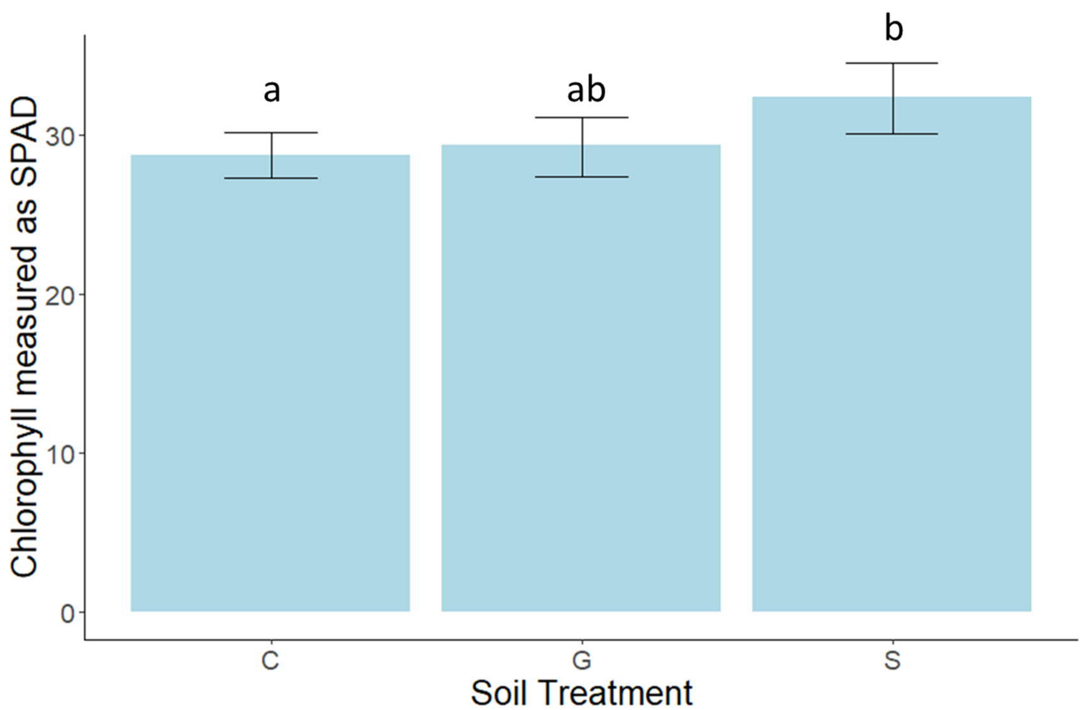

Figure 9. SPAD value was significantly different between soil treatments (C= control, G= glyphosate based herbicide, S= sterilized), but was not affected by endophyte symbiosis. Different letters indicate significant differences following Posthoc Tukey's tests (Table 4).

Table 4. Results from a Posthoc Tukey's test testing pairwise comparisons of plant parameters between the soil treatments.

Total aboveground dry biomass		**T**	**p**
Sterilized soil	Control	2.744	**0.018**
GBH	Control	1.350	0.370
GBH	Sterilized	4.050	**0.0002**
Chlorophyll (SPAD)			
Sterilized	Control	2.774	**0.017**
GBH	Control	0.461	0.899
GBH	Sterilized	2.262	0.064

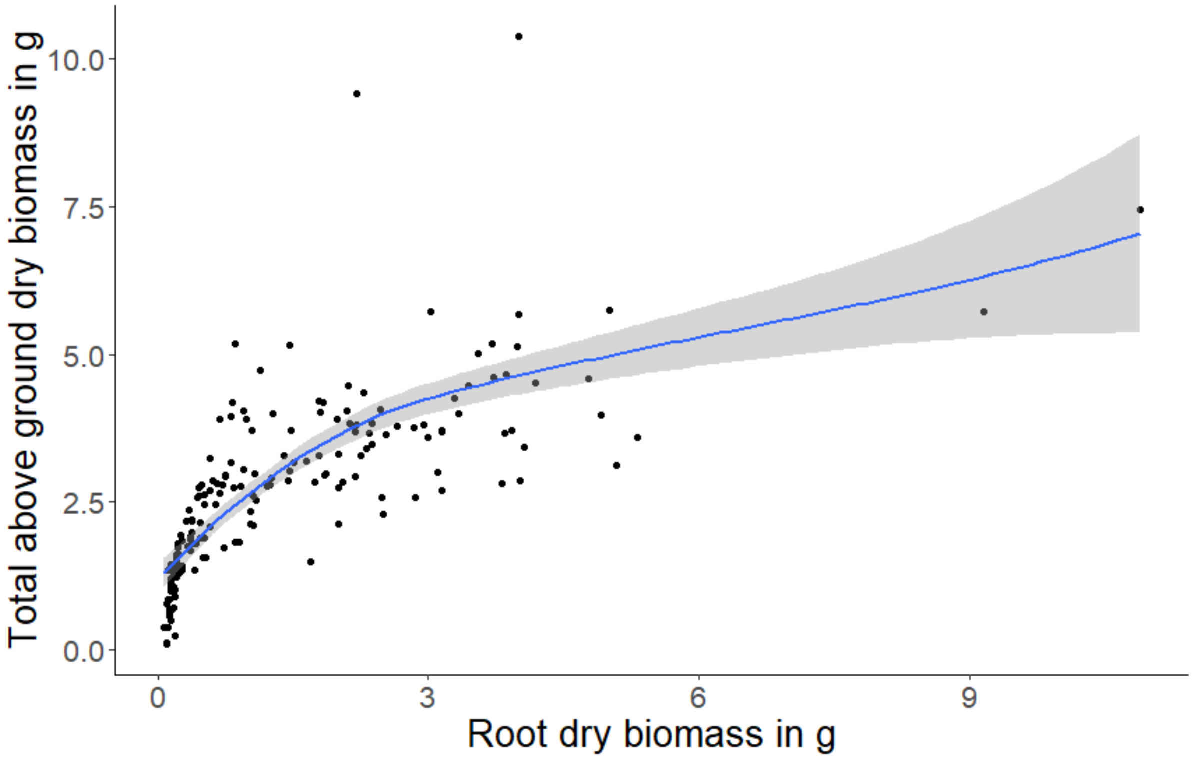

Figure 10. Correlation between root biomass and total aboveground biomass showing that higher root biomass is connected to higher aboveground plant biomass

Correlation between root biomass and total aboveground biomass shows that higher root biomass is connected to higher aboveground plant biomass (Figure 10). However, small root system can result in relatively large plants, which is displayed in the steep slope at low root biomass values.

4 Discussion

4.1 Effect of cutting height, soil treatment and endophyte status on plant parameters

My results support the hypothesis that grass management practices impact the carbon sequestration and storage potential of pastures. Cutting height significantly affected to the total above ground plant biomass, root biomass and chlorophyll content of the meadow fescues. The grasses cut to height of 5 cm produced 95.24% less total aboveground biomass, 322.23% less root biomass and had 32.98% lower chlorophyll content compared to the meadow fescues cut to 15 cm height. The uncut meadow fescues produced clearly the highest total biomass and root biomass; the uncut meadow fescues had 3.51% more total aboveground dry biomass than meadow fescues cut to 15 cm height, 50.58% more total aboveground dry biomass than meadow fescues cut to 5 cm height, and 67.48% more root biomass than meadow fescues cut to 15 cm height, and 92.30% more root biomass than meadow fescues cut to 5 cm height. Uncut meadow fescues had 37.0% higher chlorophyll content compared to the meadow fescues cut to 5 cm height, and 3.03% higher chlorophyll content compared to the meadow fescues cut to 15 cm height. My results are in concordance with my hypothesis and supported by the resource allocation hypothesis (Oesterheld 1992; Holland et al. 1996; Thornton & Millard 1996). Grasses have a capability to recover well after defoliation, but cutting too low and too often results in poor regrowth rates. After being eaten or cut, grasses need to take strength for growing from the roots, which means that part of the roots will die. If this resource allocation is experienced often, plant does not have viable roots for regrowth and it dies (Oesterheld 1992; Holland et al. 1996; Thornton & Millard 1996). Similarly to in high total aboveground biomass production in uncut plants, their chlorophyll content was high. Plants have reduced ability to capture energy and carbon needed for photosynthesis when a herbivore eats leaf tissue or if part of the plant is cut away (Gurevitch et al. 2006), which in turn leads to a decreased chlorophyll content and growth, as noticed especially with grasses cut to 5 cm height.

Perennial grasses have high organic matter content and they can mitigate greenhouse gas emissions through soil carbon sequestration and improve other ecosystem responses, water quality, nutrient cycling, and support biological diversity (Franzluebbers 2012). Cutting grasses for silage or animals grazing on pasture diminish momentarily the amount of green biomass. My results demonstrate that cutting too low leads to poor regrowth rates and small biomass. If cut or let animals to graze grass too low, it is harder for the grass to recover and it does not sequestrate as much carbon as it would when leaves are longer. Grazing intensity of pastures should be regulated to maintain adequate leaf area for maximum plant growth rates throughout the grazing season (Matches 1992).

Against my prediction endophyte status did not significantly affect the total aboveground plant biomass, root biomass and chlorophyll content of the meadow fescue grasses. Usually symbiotic endophytes offer various benefits for their hosts, for example increased growth and reproduction, and resistance to abiotic environmental stresses (Saikkonen et al. 2004; Lehtonen et al. 2005). Growing in a greenhouse might have affected the results at least partly. Because the grasses were not experiencing a competition pressure from other plants, they could use all nutrients, light energy and moisture by themselves, so endophytic fungus did not provide advantage to their host grass. In addition the beneficial effect of endophytes is often still missing in younger plants (Fuchs et al 2017b). The positive effect from the endophyte to the grass host is strongest in the environments with plenty of nutrients (Saikkonen et al. 2006; Cheplick & Faeth 2009). I did not add any fertilizers during the growing season, and during watering some nutrients might have flushed away, and that might be one reason why grasses did not benefit the positive effects of endophytes.

Meadow fescues growing in sterilized soil grew much better than grasses growing in control or glyphosate treated soil. From early on the grasses growing in the sterilized soil group were bigger and more robust than in the other groups, and they produced higher amount of total aboveground biomass and had higher chlorophyll content compared to two other groups. Grasses growing in sterilized soil had 57.98% more total aboveground dry biomass than grasses growing in control soil and 190.54% more total aboveground dry biomass than grasses growing in glyphosate based herbicide treated soil. Meadow fescues growing in sterilized soil had 20.38% more root dry biomass than grasses growing in control soil and 58.18% more root biomass than grasses growing in glyphosate based herbicide treated soil. Grasses growing in sterilized soil had 12.59% higher chlorophyll content than grasses growing in control soil and 10.23% higher chlorophyll content than grasses growing in glyphosate based herbicide treated soil. Based on these figures, having residues of glyphosate based herbicides in soil will decrease harvest of meadow fescue. In addition the death rate of grasses growing in GBH soil was elevated compared to other groups (8.3% death rate in GBH group, 1.7% death rate in control soil and 0% death rate in sterilized group). My results emphasize the importance of soil health and suggest that the use of GBH decrease the resilience of agricultural systems.

One explanation for the good growth of grasses growing in sterilized soil might be that during the sterilization in the autoclave, the killed biota has turned into a good fertilizer and has given an advantage to the grasses growing in the sterilized soil. There are examples of that on previous studies as well, where grasses performed better when growing in sterilized soil (e.g. Hines et al. 2017). Grasses can cope in poor soils, and they might not need a big microbiome around them, since they are regularly growing on yards, road verges and other nutrient poor areas, where also microbes are scarce (Rikkinen 2014; Wagg et al. 2019). In addition harmful pathogens might have lived in the soil and died during sterilization, so they did not affect to the grasses growing in sterilized soil (van der Putten & Peters 1997).

Biomass production of plants depends on energy supplied by photosynthesis. Grasses growing in sterilized soil had bigger leaf area and were able to acquire more sunlight and carbon, so they had larger amount of chlorophyll compared to control group and GBH group (even though only comparison between sterilized and control groups were significant). Glyphosate can affect the carbon sequestration and contribute to smaller leaf area and biomass. Decreased shoot and root biomass due to growing in GBH treated soil likely occurred because of decreased photosynthesis rate, disrupted growth hormone biosynthesis, or lower nutrient accumulation (Bott et al. 2008; Zobiole et al. 2010; Zobiole et al. 2012; Fuchs et al. 2021; Fuchs et al. 2022).

Correlation between root biomass and total aboveground biomass showed that higher root biomass was connected to higher aboveground plant biomass, but that small root system can result in relatively large plants. An explanation for that might be that on soils that contain relatively big amount of nutrients, like agricultural soils usually do, plants do not need to grow big root systems, because nutrients are easily available on the top soil layer.

4.2 General conclusions

Cutting grass for silage or hay and the practice how animals are grazing are underestimated when considering pasture management. My results demonstrated that it would be possible to get more harvest when increasing the cutting height or moving animals to other pasture before they have been eating the grass too low.

Soil properties may determine grass productivity. My results demonstrated that soil history of glyphosate use can determine amounts of aboveground and root biomass of grasses. Because glyphosate is harmful for the plants (Tu et al. 2001; Duke & Powles 2008; Zobiole et al. 2012; Gill et al. 2017), environment (Giesy et al. 2000; Grunewald et al. 2001; Simonsen et al. 2008; Shushkova et al. 2009; Vera et al. 2010; Battaglin et al. 2014; van Bruggen et al. 2018) and animals including humans (Daruich et al. 2001; Dallegrave et al. 2003; Richard et al. 2005; Benachour et al. 2007; Dallegrave et al. 2007; Benachour and Seralini 2009; Paganelli et al. 2010; Koller et al. 2012; Mesnage et al. 2012; Samsel & Seneff 2013; Guyton et al. 2015; Bai & Ogbourne 2016; Gill et al. 2018), it should be really thought through if it is absolutely necessary to use it. In addition some plants have developed resistance against glyphosate (Powles & Preston 2006), in which case spreading glyphosate does not have an effect to those plants.

As this is a thesis, my goals were also to learn how to design and conduct a working greenhouse experiment. Working with plants has its own benefits and restrictions, of which I wanted to learn more of. While some improvements could be done if planning a similar experiment again, I find the methods of my one-growing-season experiment appropriate and the results very interesting.

4.3 The larger scale

Rotational grazing, which means that animals are grazing in relatively small paddocks for 1-3 days, and then there is a break when the grass of that paddock can rest and grow for 20-60 days, enhances productivity compared to extensive, continuous grazing. Rotational pastures can produce almost three times as much forage as continuous pastures, and also nutritional quality has been higher on rotational pastures comparing to continuous pastures (Paine et al. 1999; Oates et al. 2011). In addition, it has been shown, that higher stocking densities (bigger amount of animals per hectare) for shorter durations are associated with greater total production per hectare than lower stocking densities. Higher stocking rate increases the nutritional value of the forage, and less pasture is wasted due to trampling, fouling, and rejection as stocking rate is high. Using rotational grazing helps to reduce feed costs and potentially increases profits by reducing overall costs of production (Fales et al. 1995; Phillip et al. 2001).

Similarly, it would be more beneficial to cut grass for silage higher than conventionally. Then grasses would have a better regrowing efficiency which increases total harvests. Also roots recover better when cutting leaves higher. That leads to the better recovery after the defoliation, less loss of plants, which means less overseeding and longer period before sowing a new pasture or silage crop (Holland et al. 1996; Thornton & Millard 1996).

Future agricultural policies need to consider how to reverse the loss of soil biodiversity. Climate change, new biotic stresses brought about by change in ecological balance, and the possible introduction of new pests, will influence the future of forage grasses. Public attitudes will require further reductions in synthetic pesticides and residues in primary products (Fletcher & Easton 1997.) Grasses can be used as a powerful carbon sink, and in addition pastures can be used to recover the biodiversity of many plant, fungus, microbe, insect, bird and mammal species. When grasses are not overgrazed, they will recover faster and more complete, they will acquire more nutrients and water with their bigger root systems, and thereby absorb more carbon from the atmosphere. Since pastures and silage fields do not need to be tilled yearly but they are perennials, they also storage more carbon in roots and soil than annual fields do. Although forests store more carbon in total compared to grasslands, in vulnerable environments driven for example periodic fires, grasslands might be better carbon storages (Dass et al. 2018). It is environmentally essential to grow pastures, because they provide important ecosystem services (for example enhanced biodiversity, prevention of erosion and nutrient runoffs, serving as carbon sinks, and food production on areas where only grasses can be grown productively) (Paustian et al. 1997; Jobbágy & Jackson 2000; Guo & Gifford 2002; Soussana et al. 2014; Poeplau et al. 2018). Pastures take around 67% of global land surface, so taking a proper care of that land would make a real difference to carbon sequestration and biodiversity.

5 Acknowledgements

I want to thank my thesis instructors Dr. Benjamin Fuchs, Dr. Marjo Helander and Dr. Kari Saikkonen for their knowledge, help and patience. They supported my work and pushed me forwards. In addition to all the guidance, I need to acknowledge the supplies, tools and materials they provided for my experiment. In addition I want to thank the Botanical Garden of Ruissalo for the space in one of the greenhouses I had for the summer.

References

Ahlholm, J. U., Helander, M., Lehtimäki, S., Wäli, P. & Saikkonen, K. (2002). *Vertically transmitted fungal endophytes: different responses of host-parasite systems to environmental conditions.* Oikos 99 (1): 173-183. https://doi.org/10.1034/j.1600-0706.2002.990118.x

Aislabie, J. & Deslippe, J. R. (2013). *Soil microbes and their contribution to soil services.* In: Edit. Dymond, J. R. Ecosystem services in New Zealand – conditions and trends. Manaaki Whenua Press, Lincoln, New Zealand. https://oldwww.landcareresearch.co.nz/__data/assets/pdf_file/0018/77040/1_12_Aislabie.pdf

Allen, E., Sheaffer, C. & Martinson, K. (2013). *Forage Nutritive Value and Preference of Cool Season Grasses under Horse Grazing.* Agronomy Journal 105 (3): 679-684. https://doi.org/10.2134/agronj2012.0300

Alward, R. D. & Joern, A. (1993). *Plasticity and overcompensation in grass responses to herbivory.* Oecologia 95: 358-364. http://www.jstor.org/stable/4220452

Bai, S.H. & Ogbourne, S. O**.** (2016). *Glyphosate: environmental contamination, toxicity and potential risks to human health via food contamination.* Environmental Science and Pollution Research 23: 18988-19001. https://link.springer.com/article/10.1007/s11356-016-7425-3

Bakker, M. M., Govers, G. & Rounsevell, M. D. A. (2004). *The crop productivity–erosion relationship: an analysis based on experimental work.* CATENA 57: 55-76. https://doi.org/10.1016/j.catena.2003.07.002.

Banks, M. L., Kennedy, A. C., Kremer, R. J. & Eivazi, F. (2014). *Soil microbial community response to surfactants and herbicides in two soils.* Applied Soil Ecology 74: 12-20. https://doi.org/10.1016/j.apsoil.2013.08.018

Bardgett, R. D. & Wardle, D. A. (2010). Aboveground-belowground linkages: biotic interactions, ecosystem processes, and global change. Oxford, UK: Oxford University Press.

Bardgett, R. D. & Wardle, D. A. (2003). *Herbivore-mediated linkages between aboveground and belowground communities.* Ecology 84 (9): 2258-2268. https://doi.org/10.1890/02-0274

Battaglin, W. A., Meyer, M. T., Kuivila, K. M. & Dietze, J. E. (2014). *Glyphosate and Its Degradation Product AMPA Occur Frequently and Widely in U.S. Soils, Surface Water, Groundwater, and Precipitation.* Journal of American Water Resources Association 50: 275-290. https://doi.org/10.1111/jawr.12159

Benachour, N. & Seralini, G. E. (2009). *Glyphosate Formulations Induce Apoptosis and Necrosis in Human Umbilical, Embryonic, and Placental Cells.* Chemical Research in Toxicology 22: 97-105. https://doi.org/10.1021/tx800218n

Benachour, N., Sipahutar, H., Moslemi, S., Gasnier, C., Travert, C. & Seralini, G. (2007). *Time- and Dose-Dependent Effects of Roundup® on Human Embryonic and Placental Cells.* Archives of Environmental Contamination and Toxicology 53: 126-133. https://link.springer.com/article/10.1007/s00244-006-0154-8

Berg, G., Gruber, M., Schloter, M. & Smalla, K. (2014). *The plant microbiome and its importance for plant and human health.* Frontiers in Microbiology 5, article 491. https://doi.org/10.3389/fmicb.2014.00491

Bergström, L., Börjesson, E. & Stenström, J. (2011). *Laboratory and Lysimeter Studies of Glyphosate and Aminomethylphosphonic Acid in a Sand and a Clay Soil.* Journal of Environmental Quality 40 (1): 98-108. https://doi.org/10.2134/jeq2010.0179

Bolinder, M. A., Angers, D. A., Bélanger, G., Michaud, R. & Laverdière, M. R. (2002). *Root biomass and shoot to root ratios of perennial forage crops in eastern Canada.* Canadian Journal of Plant Science 82 (4): 731-737. doi:10.4141/p01-139

Borggaard, O.K. & Gimsing, A. L. (2008). *Fate of Glyphosate in Soil and the Possibility of Leaching to Ground and Surface Waters: A Review.* Pest Management Science 64: 441-456. https://doi.org/10.1002/ps.1512

Bott, S., Tesfamariam, T., Candan, H., Cakmak, I., Römheld, V. & Neumann, G. (2008). *Glyphosate-induced impairment of plant growth and micronutrient status in glyphosate-resistant soybean (Glycine max L.).* Plant and Soil 312 (1-2): 185-194. doi:10.1007/s11104-008-9760-8

Bringolf, R. B., Cope, W. G., Mosher, S., Barnhart, M. C. & Shea, D. (2007). *Acute and Chronic Toxicity of Glyphosate Compounds to Glochidia and Juveniles of Lampsilis Siliquoidea*

(Unionidae). Environmental Toxicology and Chemistry 26 (10): 2094-2100. https://doi.org/10.1897/06-519R1.1

Brink, G. E., Casler, M. D. & Martin, N. P. (2010). *Meadow Fescue, Tall Fescue, and Orchardgrass Response to Defoliation Management.* Agronomy Journal 102 (2): 667-674. doi:10.2134/agronj2009.0376

Briske, D. D., Boutton, T. W. & Wang, Z. (1996). *Contribution of flexible allocation priorities to herbivory tolerance in C4 perennial grasses: an evaluation with 13C labeling.* Oecologia 105 (2): 151-159. doi:10.1007/bf00328540

Brown, S. & Lugo, A. E. (1990). *Effects of forest clearing and succession on the carbon and nitrogen content of soils in Puerto Rico and US Virgin Islands.* Plant and Soil 124 (1): 53-64. http://www.jstor.org/stable/42938486

Bulgarelli D., Schlaeppi K., Spaepen S., Loren Ver, van Themaat E. & Schulze-Lefert P. (2013). *Structure and functions of the bacterial microbiota of plants.* Annual Review of Plant Biology 64: 807-838. https://www.annualreviews.org/doi/full/10.1146/annurev-arplant-050312-120106

Cagnano, G., Lenk, I., Roulund, N., Jensen, C.S., Cox, M.P. & Asp, T. (2020). *Mycelial biomass and concentration of loline alkaloids driven by complex population structure in Epichloë uncinata and meadow fescue (Schedonorus pratensis).* Mycologia 112 (3): 474-490. https://doi.org/10.1080/00275514.2020.1746607

Cheplick, G.P. & Faeth, S. (2009). *Ecology and evolution of the grass-endophyte symbiosis.* Oxford University Press, New York. doi:10.1093/acprof:oso/9780195308082.001.0001

Dallegrave, E., Mantese, F. D., Coelho, R. S., Pereira, J. D., Dalsenter, P. R. & Langeloh, A. (2003). *The Teratogenic Potential of the Herbicide Glyphosate-Roundup in Wistar Rats.* Toxicology Letters 142 (1-2): 45-52. https://doi.org/10.1016/S0378-4274(02)00483-6

Dallegrave, E., Mantese, F. D., Oliveira, R. T., Andrade,A. J. M., Dalsenter, P. R. & Langeloh, A. (2007). *Pre- and Postnatal Toxicity of the Commercial Glyphosate Formulation in Wistar Rats.* Archives of Toxicology 81 (9): 665-673. DOI 10.1007/s00204-006-0170-5

Daruich, J., Zirulnik, F. & Gimenez, M. S. (2001). *Effect of the Herbicide Glyphosate on Enzymatic Activity in Pregnant Rats and Their Foetuses.* Environmental Research 85 (3): 226-231. https://doi.org/10.1006/enrs.2000.4229

Dass, P., Houlton, B. Z., Wang, Y. & Warlind, D. (2018). *Grasslands may be more reliable carbon sinks than forests in California.* Environmental Research Letters 13 (7): 074027. doi:10.1088/1748-9326/aacb39

Dirihan, S., Helander, M.L., Saloniemi, I., Gundel, P.E. & Saikkonen, K. (2014). *Effects of systemic fungal endophytes on the performance of meadow fescue and tall fescue in mixtures with red clover.* Grass and Forage Science 70 (3): 465-473. https://doi.org/10.1111/gfs.12145

Doetterl, S., Berhe, A. A., Nadeu, E., Wang, Z., Sommer, M. & Fiener, P. (2016). *Erosion, deposition and soil carbon: A review of process-level controls, experimental tools and models to address C cycling in dynamic landscapes.* Earth-Science Reviews 154: 102-122. doi:10.1016/j.earscirev.2015.12.0

Duke, S. O. & Powles, S. B. (2008). *Glyphosate: a once-in-a-century herbicide.* Pest Management Science 64 (4): 319-325. https://doi.org/10.1002/ps.1518

Edginton, A. N., Sheridan, P. M., Stephenson, G. R., Thompson, D. G. & Boermans, H. J. (2004). *Comparative Effects of pH and Vision® on Two Life Stages of Four Anuran Amphibian Species.* Environmental Toxicology and Chemistry 23 (4): 815-822. https://doi.org/10.1897/03-115

Fales, S. L., Muller, L. D., Ford, S. A., O'Sullivan, M., Hoover, R. J., Holden, L. A., Lanyon, L. E. & Buckmaster, D. R. (1995). *Stocking Rate Affects Production and Profitability in a Rotationally Grazed Pasture System.* Journal of Production Agriculture 8 (1): 88-96. doi:10.2134/jpa1995.0088

FAO. (2020). *Land use in agriculture by the numbers.* Sustainable Food and Agriculture. https://www.fao.org/sustainability/news/detail/en/c/1274219/#:~:text=Globally%20agricultural%20land%20area%20is,and%20pastures)%20for%20grazing%20livestock. [Seen 12.4.2022]

Ferraro, D. O. & Oesterheld, M. (2002). *Effect of defoliation on grass growth. A quantitative review.* Oikos 98 (1): 125-133. doi:10.1034/j.1600-0706.2002.980113.x

Fjellheim, S. & Rognli, O.-A. (2005). *Genetic Diversity within and among Nordic Meadow Fescue (Festuca pratensis Huds.) Cultivars Determined on the Basis of AFLP Markers.* Crop Science 45 (5): 2081-2086. https://doi.org/10.2135/cropsci2005.0091

Fletcher, L. R. & Easton, H. S. (1997). *The Evaluation and Use of Endophytes for Pasture Improvement.* In: Bacon, C. W. & Hill, N. S (edit.) Neotyphodium/Grass Interactions, p. 209-227. doi:10.1007/978-1-4899-0271-9_39

Foreca. (2021). Täsmäsää. Turku, Artukainen. https://www.foreca.fi/Finland/Turku

Franzluebbers, A. J. (2012). *Grass roots of soil carbon sequestration*. Carbon Managenment 3 (1): 9-11. https://doi.org/10.4155/cmt.11.73

Fuchs, B., Krischke, M., Mueller, M. J. & Krauss, J. (2017a). *Herbivore-specific induction of defence metabolites in a grass-endophyte association.* Functional Ecology 31 (2): 318-324. doi:10.1111/13652435.12755

Fuchs, B., Krischke, M., Mueller, M. J. & Krauss, J. (2017b). *Plant age and seasonal timing determine endophyte growth and alkaloid biosynthesis.* Fungal Ecology 29: 52-58. doi:10.1016/j.funeco.2017.06.003

Fuchs, B., Laihonen, M., Muola, A., Saikkonen, K., Dobrev, P. I., Vankova, R. & Helander, M. (2022). *A Glyphosate-Based Herbicide in Soil Differentially Affects Hormonal Homeostasis and Performance of Non-target Crop Plants.* Frontiers in Plant Science 12: 787958. doi: 10.3389/fpls.2021.787958.

Fuchs, B., Saikkonen, K. & Helander, M. (2011). *Glyphosate-modulated biosynthesis driving plant defense and species interactions.* Trends in Plant Science 26: 312-323. doi:10.1016/j.tplants.2020.11.004

Georgiadis, N. J., Ruess, R. W., McNaughton, A. J. & Western, D. (1989). *Ecological conditions that determine when grazing stimulate grass production.* Oecologia 81: 316-322. https://doi.org/10.1007/BF00377077

Giesy, J. P., Dobson, S. & Solomon, K. R. (2000). *Ecotoxicological Risk Assessment for Roundup® Herbicide.* Reviews of Environmental Contamination and Toxicology 167: 35-120. doi:10.1007/978-1-4612-1156-3_2

Gill J. P. K., Sethi N. & Mohan, A. (2017). *Analysis of the glyphosate herbicide in water, soil and food using derivatising agents.* Environmental Chemistry Letters 15 (1): 85-100. https://link.springer.com/article/10.1007/s10311-016-0585-z

Gill, J. P. K., Sethi, N., Mohan, A., Datta, S. & Girdhar, M. (2018). *Glyphosate toxicity for animals.* Environmental Chemistry Letters 16**:** 401–426. https://link.springer.com/article/10.1007/s10311-017-0689-0

Gomes, M. P., Gingras Le Manac'h, S., Maccario, S., Labrecque, M., Lucotte, M. & Juneau, P. (2016). *Differential effects of glyphosate and aminomethylphosphonic acid (AMPA) on photosynthesis and chlorophyll metabolism in willow plants.* Pesticide Biochemistry and Physiology 130: 65-70. https://doi.org/10.1016/j.pestbp.2015.11.010

Grunewald, K., Schmidt, W., Unger, C. & Hanschmann, G. (2001). *Behavior of Glyphosate and Aminomethylphosphonic Acid (AMPA) in Soils and Water of Reservoir Radeburg II Catchment (Saxony/Germany).* Journal of Plant Nutrition and Soil Science 164 (1): 65-70. https://doi.org/10.1002/1522-2624(200102)164:1<65::AID-JPLN65>3.0.CO;2-G

Gullap, M. K., Erkovan, H. I. & Koc, A. (2011). *The effect of bovine saliva on growth attributes and forage quality of two contrasting cool season perennial grasses grown in three soils of different fertility.* The Rangeland Journal 33 (3): 307. doi:10.1071/rj10063

Guo, L. B. & Gifford, R. M. (2002). *Soil carbon stocks and land use change: a meta-analysis.* Global Change Biology 8 (4): 345-360. doi:10.1046/j.1354-1013.2002.00486.x

Gurevitch, J., Scheiner, S. & Fox, G. A. (2006). The Ecology of Plants. 2nd edition. Sinauer Associates, Massachussetts, USA.

Guyton, K. Z., Loomis, D., Grosse, Y., Ghissassi, F. E., Benbrahim-Tallaa, L., Guha, N., Scoccianti, C., Matlock, H., Straif, K. (2015). *Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate.* Lancet Oncology 16 (5): 490-491. https://doi.org/10.1016/S1470-2045(15)70134-8

Helander, M., Pauna, A., Saikkonen, K. & Saloniemi, I. (2019). *Glyphosate residues in soil affect crop plant germination and growth.* Scientific Reports 9 (1): 19653. doi:10.1038/s41598-019-56195-3

Hewitt, K. G., Mace, W. J., McKenzie, C. M., Matthew, C. & Popay, A. J. (2020). *Fungal Alkaloid Occurrence in Endophyte-Infected Perennial Ryegrass during Seedling Establishment.* Journal of Chemical Ecology 46: 410-421. https://doi.org/10.1007/s10886-020-01162-w

Hines, J., Pabst, S., Mueller, K. E., Blumenthal, D. M., Cesarz, S. & Eisenhauer, N. (2017). *Soil-mediated effects of global change on plant communities depend on plant growth form.* Ecosphere 8 (11). https://doi.org/10.1002/ecs2.1996

Holland, J. N., Cheng, W. X. & Crossley, D. A. (1996). *Herbivore-induced changes in plant carbon allocation: assessment of belowground C fluxes using carbon-14.* Oecologia 107 (1): 87-94. https://www.jstor.org/stable/4221310

Huitu, O., Forbes, K. M., Helander, M., Julkunen-Tiitto, R., Lambin, X., Saikkonen, K., Stuart, P., Sulkama, S. & Hartley, S. (2014). *Silicon, endophytes and secondary metabolites as grass defenses against mammalian herbivores.* Frontiers in Plant Science 5. doi:10.3389/fpls.2014.00478

Hume, D.E., Cooper, B.M. & Panckhurst, K.A. (2009). *The role of endophyte in determining the persistence and productivity of ryegrass, tall fescue and meadow fescue in Northland.* Proceedings of the New Zealand Grassland Association 71 (1): 145-150 . https://doi.org/10.33584/jnzg.2009.71.2754

Jensen, J.G., Popay, A.J. & Tapper, B.A. (2009). *Argentine stem weevil adults are affected by meadow fescue endophyte and its loline alkaloids.* New Zealand Plant Protection 62 (1): 12-18. https://doi.org/10.30843/nzpp.2009.62.4800

Jobbágy, E. G. & Jackson, R. B. (2000). *The Vertical Distribution of Soil Organic Carbon and Its Relation to Climate and Vegetation.* Ecological Applications 10 (2): 423-436. doi:10.2307/2641104

Kirkels, F. M. S. A., Cammeraat, L. H. & Kuhn, N. J. (2014). *The fate of soil organic carbon upon erosion, transport and deposition in agricultural landscapes — A review of different concepts.* Geomorphology 226: 94-105. https://doi.org/10.1016/j.geomorph.2014.07.023

Koller, V. J., Fürhacker, M., Nersesyan, A., Mišík, M., Eisenbauer, M. & Knasmueller, S. (2012). *Cytotoxic and DNA-damaging properties of glyphosate and Roundup in human-derived buccal epithelial cells.* Archives of Toxicology 86: 805-813. https://link.springer.com/article/10.1007/s00204-012-0804-8

Kremer, R., Means, N. & Kim, S. (2005). *Glyphosate affects soybean root exudation and rhizosphere micro-organisms.* International Journal of Environmental Analytical Chemistry 85 (15): 1165-1174. https://doi.org/10.1080/03067310500273146

Kremer, R. J. & Means, N. E. (2009). *Glyphosate and glyphosate-resistant crop interactions with rhizosphere microorganisms.* European Journal of Agronomy 31 (3): 153-161. https://doi.org/10.1016/j.eja.2009.06.004

Kuklinsky-Sobral, J., Araújo, W. L., Mendes, R., Pizzirani-Kleiner, A. A. & Azevedo, J. L. (2005). *Isolation and characterization of endophytic bacteria from soybean (Glycine max) grown in soil treated with glyphosate herbicide.* Plant Soil 273: 91-99. https://link.springer.com/article/10.1007/s11104-004-6894-1

LaCanne, C. E. & Lundgren, J. G. (2018). *Regenerative agriculture: merging farming and natural resource conservation profitably.* PeerJ 6. https://peerj.com/articles/4428/?td=tw

Lange, M., Eisenhauer, N., Sierra, C. A., Bessler, H., Engels, C., Griffiths, R. I., Mellado-Vázquez, P. G., Malik, A. A., Roy, J., Scheu, S., Steinbeiss, S., Thomson, B. C., Trumbore, S. C. & Gleixner, G. (2015). *Plant diversity increases soil microbial activity and soil carbon storage.* Nature Communications 6 (1): 6707. https://doi.org/10.1038/ncomms7707

Lehtonen, P. T., Helander, M., Siddiqui, S. A., Lehto, K. & Saikkonen, K. (2006). *Endophytic fungus decreases plant virus infections in meadow ryegrass (Lolium pratense).* Biology Letters 2 (4): 620-623. doi:10.1098/rsbl.2006.0499n

Lehtonen, P., Helander, M., Wink, M., Sporer, F. & Saikkonen, K. (2005). *Transfer of endophyte-origin defensive alkaloids from a grass to a hemiparasitic plant.* Ecology Letters 8 (12): 1256-1263. doi:10.1111/j.1461-0248.2005.00834.x

Leifeld, J. & Fuhrer, J. (2010). *Organic Farming and Soil Carbon Sequestration: What Do We Really Know About the Benefits?* AMBIO 39 (8): 585-599. doi:10.1007/s13280-010-0082-8

Leino, L., Tall, T., Helander, M., Saloniemi, I., Saikkonen, K., Ruuskanen, S. & Puigbò, P. (2020). *Classification of the glyphosate target enzyme (5-enolpyruvylshikimate-3-phosphate synthase) for assessing sensitivity of organisms to the herbicide*. Journal of Hazardous Materials 124556. doi:10.1016/j.jhazmat.2020.124556

Luonnonvarakeskus. (2019). Kasvinsuojeluaineiden käyttö valmisteryhmittäin. Tilastotietokanta. https://statdb.luke.fi/PXWeb/pxweb/fi/LUKE/LUKE__02%20Maatalous__04%20Tuotanto__34%20Kasvinsuojeluaineiden%20kaytto%20maataloudessa/01_Kasvinsuojeluainekaytto.px/table/tableViewLayout2/?loadedQueryId=cf708da9-ee8d-4124-9f6d-fd8a80b34ee0&timeType=from&timeValue=2013 [Seen 23.3.2022]

Malinowski, D., Leuchtmann, A., Schmidt, D. & Nösberger, J. (1997). *Symbiosis with Neotyphodium uncinatum Endophyte May Increase the Competitive Ability of Meadow Fescue.* Agronomy Journal 89 (5): 833-839. doi:10.2134/agronj1997.00021962008900050019x

Malinowski, D. P. & Belesky, D. P. (2000). *Adaptations of Endophyte-Infected Cool-Season Grasses to Environmental Stresses: mechanisms of drought and mineral stress tolerance.* Crop Science 40 (4): 923-940. doi:10.2135/cropsci2000.404923x

Malinowski, D. P. & Belesky, D. P. (1999). *Tall fescue aluminum tolerance is affected by* Neotyphodium coenophialum endophyte. Journal of Plant Nutrition 22 (8): 1335-1349. https://www.tandfonline.com/doi/abs/10.1080/01904169909365716

Mariotte, P., Mehrabi, Z., Bezemer, T. M., De Deyn, G. B., Kulmatiski, A., Drigo, B., Veen, G. F., van der Heijden, M. G. A. & Kardol, P. (2018). *Plant–soil feedback: bridging natural and agricultural sciences.* Trends in Ecology & Evolution 33 (2): 129-142. https://doi.org/10.1016/j.tree.2017.11.005

Matches, A. G. (1992). *Plant Response to Grazing: A Review.* Journal of Production Agriculture 5 (1): 1-7. doi:10.2134/jpa1992.0001

McNaughton, S. J. (1992). *Laboratory-simulated grazing: interactive effects of defoliation and canopy closure on Serengeti grasses.* Ecology 73 (1): 170-182. doi:10.2307/1938729

McNaughton, S. J., Wallace, L. & Coughenour, M. B. (1983). *Plant adaptation in an ecosystem context: effects of defoliation, nitrogen, and water on growth of an African C4 sedge.* Ecology 64 (2): 307-318. https://doi.org/10.2307/1937078

Mesnage, R., Bernay, B. & Seralini, G. E. (2012). *Ethoxylated Adjuvants of Glyphosate-Based Herbicides Are Active Principles of Human Cell Toxicity.* Toxicology 313 (2-3): 122-128. https://doi.org/10.1016/j.tox.2012.09.006

Moore, L.J., Fuentes, L., Rodgers, J. H., Bowerman, W. W., Yarrow, G. K., Chao, W.Y. & Bridges, W. C. (2012). *Relative Toxicity of the Components of the Original Formulation of Roundup® to Five North American Anurans.* Ecotoxicology and Environmental Safety 78: 128-133. https://doi.org/10.1016/j.ecoenv.2011.11.025

Morse, L., Faeth, S. & Day, T. (2007). *Neotyphodium interactions with a wild grass are driven mainly by endophyte haplotype.* Functional ecology 21 (4): 813-822. https://doi.org/10.1111/j.1365-2435.2007.01285.x

Oates, L. G., Undersander, D. J., Gratton, C., Bell, M. M. & Jackson, R. D. (2011). *Management Intensive Rotational Grazing Enhances Forage Production and Quality of Sub-humid Cool Season Pastures*. Crop Science 51 (2): 892-901. https://doi.org/10.2135/cropsci2010.04.0216

Okada, E., Costa, J. L. & Bedmar, F. (2016). *Adsorption and mobility of glyphosate in different soils under no-till and conventional tillage.* Geoderma 263: 78-85. https://doi.org/10.1016/j.geoderma.2015.09.009

Oesterheld, M. (1992). *Effect of defoliation intensity on aboveground and belowground relative growth rates.* Oecologia 92 (3): 313-316. https://doi.org/10.1007/BF00317456

Oesterheld, M. and McNaughton, S. J. (1988). *Intraspecific variation in the response of Themeda triandra to defoliation: the effect of time of recovery and growth rates on compensatory growth.* Oecologia 77 (2): 181-186. https://www.jstor.org/stable/4218759

O'Mara, F. P. (2012). *The role of grasslands in food security and climate change.* Annals of Botany 110 (6): 1263-1270. doi:10.1093/aob/mcs209

Paganelli, A., Gnazzo, V., Acosta, H., Lopez, S. L. & Carrasco, A. E. (2010). *Glyphosate-Based Herbicides Produce Teratogenic Effects on Vertebrates by Impairing Retinoic Acid Signalling.* Chemical Research in Toxicology 23 (10): 1586-1595. https://doi.org/10.1021/tx1001749

Paine, L. K., Undersander, D. & Casler, M. D. (1999). *Pasture Growth, Production, and Quality Under Rotational and Continuous Grazing Management.* Journal of Production Agriculture 12 (4): 569-577. https://doi.org/10.2134/jpa1999.0569

Patchett, B., Gooneratne, R., Chapman, B. & Fletcher, L. (2011). *Effects of loline-producing endophyte-infected meadow fescue ecotypes on New Zealand grass grub (Costelytra zealandica).* New Zealand Journal of Agricultural Research 54 (4): 303-313. https://doi.org/10.1080/00288233.2011.608686

Paustian, K., Collier, S., Baldock, J., Burgess, R., Creque, J., DeLonge, M., Dungait, J., Ellert, B., Frank, S., Goddard, T., Govaerts, B., Grundy. M., Henning, M., Izaurralde, R. C., Madaras, M., McConkey, B., Porzig, E., Rice, C., Searle, R., Seavy, N., Skalsky, R., Mulhern, W. & Jahn, M. (2019). *Quantifying carbon for agricultural soil management: from the current status toward a global soil information system*. Carbon Managenment 10 (6): 567-587. https://doi.org/10.1080/17583004.2019.1633231

Paustian, K., Collins, H. P. & Paul, E. A. (1997). *Management controls on soil carbon*. In: *Soil organic matter in temperate agroecosystems: Long-term experiments in North America.* (Paul, E. A., Paustian, K., Elliott, E. T. & Cole, C. V. eds.), p. 15-49. CRC Press, New York.

Phillip, L. E., Goldsmith, P., Bergeron, M. & Peterson, P. R. (2001).*Optimizing pasture management for cow-calf production: the roles of rotational frequency and stocking rate in the context of system efficiency.* Canadian Journal of Animal Science 81 (1): 47-56. doi:10.4141/a00-044

Pieterse, C. M. J., de Jonge, R. & Berendsen, R. L. (2016). *The soil-borne supremacy.* Trends in Plant Science 21: 171-173. https://doi.org/10.1016/j.tplants.2016.01.018

Pineiro, G., Paruelo, J. M., Oesterheld, M. & Jobbagy, E. G. (2010). *Pathways of grazing effects on soil organic carbon and nitrogen.* Rangeland Ecology & Management 63 (1): 109-119. https://doi.org/10.2111/08-255.1

Poeplau, C., Zopf, D., Greiner, B., Geerts, R., Korvaar, H., Thumm, U., Don, M., Heidkamp, A. & Flessa, H. (2018). *Why does mineral fertilization increase soil carbon stocks in temperate grasslands?* Agriculture, Ecosystems & Environment 265:144-155. https://doi.org/10.1016/j.agee.2018.06.003

Popay, A. & Tapper, B. (2007). *Endophyte effects on consumption of seed and germinated seedlings of ryegrass and fescue by grass grub (Costelytra zealandica) larvae.* In: Edit. A. J. Popay & E. R. Thom. 6th International Symposium on Fungal Endophytes of Grasses. Grassland Research and Practice Series 13: 353-356. Dunedin, New Zealand. New

Zealand Grassland Association.

Popay, A., Tapper, B. & Podmore, C. (2009). *Endophyte-infected meadow fescue and loline alkaloids affect Argentine stem weevil larvae.* New Zealand Plant Protection 62 (1): 19-27. doi: 10.30843/nzpp.2009.62.4801.

Powles, S. B. & Preston, C. (2006). *Evolved Glyphosate Resistance in Plants: Biochemical and Genetic Basis of Resistance.* Weed Technology 20 (2): 282-289. doi:10.1614/WT-04-142R.1

Raaijmakers, J. M. & Mazzola, M. (2016). *Soil immune responses*. Science 352 (6292): 1392-1393. DOI: 10.1126/science.aaf3252

Rao, K. M. & Rognli, O. A. (2014). *Meadow fescue.* In: Edit. H. Cai, T. Yamada & C. Kole: Genetics, Genomics and Breeding of Forage Crops. CRC Press, Boca Raton, US. 90-104.

Rasmussen, S., Parsons, A. J., Fraser, K., Xue, H. & Newman, J. A. (2008). *Metabolic profiles of Lolium perenne are differentially affected by nitrogen supply, carbohydrate content, and fungal endophyte infection.* Plant Physiology 146 (3): 1440-1453. doi:10.1104/pp.107.111898

Reardon, P. O., Leinweber, C. L. & Merrill, L. B. (1974). *Response of Sideoats Grama to Animal Saliva and Thiamine.* Journal of Range Management 27 (5): 400-401. doi:10.2307/3896502

Richard, S., Moslemi,S., Sipahutar, H., Benachour, N. & Seralini, G. (2005). *Differential Effects of Glyphosate and Roundup® on Human Placental Cells and Aromatase.* Environmental Health Perspectives 113 (6): 716-720. https://doi.org/10.1289/ehp.7728

Rikkinen, J. (2014). Heinät ja sarat Suomen luonnossa. Kustannusosakeyhtiö Otava. Helsinki.

Saari, S., Helander, M., Lehtonen, P., Wallius, E. & Saikkonen, K. (2010). *Fungal endophytes reduce regrowth and affect competitiveness of meadow fescue in early succession of pastures.* Grass and Forage Science 65 (3): 287-295. https://doi.org/10.1111/j.1365-2494.2010.00746.x

Sabzalian, M. R. & Mirlohi, A. (2010). *Neotyphodium endophytes trigger salt resistance in tall and meadow fescues.* Journal of Plant Nutrition and Soil Science 173 (6): 952-957. doi:10.1002/jpln.200900345

Saikkonen, K., Lehtonen, P., Helander, M., Koricheva, J. & Faeth, S. H. (2006). *Model systems in ecology: dissecting the endophyte-grass literature.* Trends in Plant Science 11 (9): 428-433. DOI: 10.1016/j.tplants.2006.07.001

Saikkonen, K., Ruokolainen, K., Huitu, O., Gundel, P. E., Piltti, T., Hamilton, C. E. & Helander, M. (2013). *Fungal endophytes help prevent weed invasions.* Agriculture, Ecosystems & Environment 165: 1-5. https://doi.org/10.1016/j.agee.2012.12.002

Saikkonen, K., Wäli, P., Helander, M. & Faeth, S. H. (2004). *Evolution of endophyte–plant symbioses.* Trends in Plant Science 9 (6): 275-280. https://doi.org/10.1016/j.tplants.2004.04.005

Saikkonen, K., Wäli, P. R., & Helander, M. (2010). *Genetic Compatibility Determines Endophyte-Grass Combinations.* PLoS ONE 5 (6): e11395. doi:10.1371/journal.pone.0011395

Samsel, A. & Seneff, S. (2013). *Glyphosate's Suppression of Cytochrome P450 Enzymes and Amino Acid Biosynthesis by the Gut Microbiome: Pathways to Modern Diseases.* Entropy 15 (4): 1416-1463. https://doi.org/10.3390/e15041416

Schardl, C. L., Grossman, R. B., Nagabhyru, P., Faulkner, J. R. & Mallik, U. P. 2007. Loline alkaloids: Currencies of mutualism. Phytochemistry 68 (7): 980-996. doi:10.1016/j.phytochem.2007.01.010

Scharlemann, J. P. W., Tanner, E. V. J., Hiederer, R. & Kapos, V. (2014). *Global soil carbon: understanding and managing the largest terrestrial carbon pool*. Carbon Managenment 5 (1): 81-91. https://doi.org/10.4155/cmt.13.77

Shushkova, T., Ermakova, I. & Leontievsky, A. (2009). *Glyphosate bioavailability in soil.* Biodegradation 21: 403-410. https://link.springer.com/article/10.1007/s10532-009-9310-y

Sihtmäe, M., Blinova, I., Künnis-Beres, K., Kanarbik, L., Heinlaan, M. & Kahru, A. (2013). *Ecotoxicological effects of different glyphosate formulations.* Applied Soil Ecology 72: 215-224. https://doi.org/10.1016/j.apsoil.2013.07.005

Simonsen, L., Fomsgaard, I. S., Svensmark, B. & Spliid, N. H. (2008). *Fate and availability of glyphosate and AMPA in agricultural soil.* Journal of Environmental Science and Health, Part B, Pesticides, Food Contaminants, and Agricultural Wastes 43 (5): 365-375. https://doi.org/10.1080/03601230802062000

Sitters, J., Wubs, E. R. J., Bakker, E. S., Crowther, T. W., Adler, P. B., Bagchi, S., Bakker, J. D., Biederman, L., Borer, E. T., Cleland, E. E., Eisenhauer, N., Firn, J., Gherardi, L., Hagenah, N., Hautier, Y., Hobbie, S. E., Knops, J. M. H., MacDougall, A. S., McCulley, R. L., Moore, J. L., Mortensen, B., Peri, P. L., Prober, S. M., Riggs, C., Risch, A. C., Schütz, M., Seabloom, E. W., Siebert, J., Stevens, C. J. & Veen, G. F. (Ciska). (2020). *Nutrient availability controls the impact of mammalian herbivores on soil carbon and nitrogen pools in grasslands.* Global Change Biology 26 (4):2060-2071. doi:10.1111/gcb.15023

Sokol, N. W. & Bradford, M. A. (2019). *Microbial formation of stable soil carbon is more efficient from belowground than aboveground input.* Nature Geoscience 12 (1): 46-53. https://doi.org/10.1038/s41561-018-0258-6

Soussana, J. F., Klumpp, K. & Ehrhardt, F. (2014). *The role of grassland in mitigating climate change.* 25th General Meeting of the European Grassland Federation, European Grassland Federation (EGF). Aberystwyth, United Kingdom. Grassland Science in Europe 19: 75-90. https://hal.inrae.fr/hal-02738557/document

Spiering, M. J., Lane, G. A., Christensen, M. J. & Schmid, J. (2005). *Distribution of the fungal endophyte Neotyphodium lolii is not a major determinant of the distribution of fungal alkaloids in Lolium perenne plants.* Phytochemistry 66 (2): 195-202. https://doi.org/10.1016/j.phytochem.2004.11.021

St. Laurent, A., Merwin, I. A. & Thies, J. E. (2008). *Long-term orchard groundcover management systems affect soil microbial communities and apple replant disease severity.* Plant Soil 304: 209-225. https://link.springer.com/article/10.1007/s11104-008-9541-4

Sviridov, A. V., Shushkova, T. V., Ermakova, I. T., E. V. Ivanova, E. V., D. O. Epiktetov, D. O. & Leontievsky, A. A. (2015). *Microbial degradation of glyphosate herbicides (Review).* Applied Biochemistry and Microbiology 51: 188-195. https://link.springer.com/article/10.1134/S0003683815020209

Takai, T., Sanada, Y. & Yamada, T. (2010). I*nfluence of the fungal endophyte Neotyphodium uncinatum on the persistency and competitive ability of meadow fescue (Festuca pratensis Huds.).* Grassland Science 56 (2): 59-64. doi:10.1111/j.1744-697x.2010.00175.x

Thornton, B. & Millard, P. (1996). *Effects of severity of defoliation on root functioning in grasses.* Journal of Range Management 49 (5): 443-447. https://doi.org/10.2307/4002927

Tsiafouli, M. A., Thébault, E., Sgardelis, S. P., de Ruiter, P. C., van der Putten, W. H., Birkhofer, K., Hemerik, L., de Vries, F. T., Bardgett, R. D., Brady, M. V., Bjornlund, L., Jørgensen, H. B., Christensen, S., d'Hertefeldt, T., Hotes, S., Gera Hol, W. H., Frouz, J., Liiri, M., Mortimer, S.R., Setälä, H., Tzanopoulos, J., Uteseny, K., Pižl, V., Stary, J., Wolters, V. & Hedlund, K. (2014). *Intensive agriculture reduces soil biodiversity across Europe.* Global Change Biology 21 (2): 973-985. doi:10.1111/gcb.12752

Tu, M., Hurd, C. & Randall, J. M. (2001). *Weed control methods handbook: Tools & Techniques for Use in Natural Areas.* The Nature Conservancy 7E.1-7E.10. All U.S. Government Documents, Utah Regional Depository. Paper 533. https://digitalcommons.usu.edu/govdocs/533

Uusi-Kämppä, J. (2019). *Glyfosaatti häviää hitaasti suomalaisesta viljelymaasta.* Luonnonvarakeskus. https://www.luke.fi/uutinen/glyfosaatti-haviaa-hitaasti-suomalaisesta-viljelymaasta/#:~:text=%E2%80%93%20Maahan%20nopeasti%20sitoutuva%20glyfosaatti%20ja,Luken%20erikoistutkija%20Jaana%20Uusi%2DK%C3%A4mpp%C3%A4. [Seen 23.3.2022]

Vail, S. G. (1992). *Selection for overcompensatory plant responses to herbivory: a mechanism for the evolution of plant-herbivore mutualism.* The American Naturalist 139 (1): 1-8. https://www.jstor.org/stable/2462581

van Bruggen, A. H. C., He, M. M., Shin, K., Mai, V., Jeong, K. C., Finckh, M. R. & Morris, J. G. Jr. (2018). *Environmental and health effects of the herbicide glyphosate.* Science of The Total Environment 616-617: 255-268. https://doi.org/10.1016/j.scitotenv.2017.10.309

van Bruggen, A. H. C., Narouei Khandan, H. A., Gravel, V. & Blok, W. J. (2016). *Corky root severity, root knot nematode galling and microbial communities in soil, rhizosphere and rhizoplane in organic and conventional greenhouse compartments.* Applied Soil Ecology 100: 112-123. https://doi.org/10.1016/j.apsoil.2015.11.015

van Bruggen, A. H. C., Sharma, K., Kaku, E., Karfopoulos, S., Zelenev, V. V. & Blok, W. J. (2015). *Soil health indicators and Fusarium wilt suppression in organically and conventionally managed greenhouse soils.* Applied Soil Ecology 86: 192-201. https://doi.org/10.1016/j.apsoil.2014.10.014

van der Putten, W. H. & Peters, B. A. M. (1997). *How Soil-Borne Pathogens May Affect Plant Competition.* Ecology, 78(6): 1785. doi:10.2307/2266101

Vera, M. S., Lagomarsino, L., Sylvester, M., Perez, G. L., Rodriguez, P., Mugni, H., Sinistro, R., Ferraro, M., Bonetto, C., Zagarese, H. & Pizarro, H. (2010). *New Evidence of Roundup® (glyphosate formulation) Impact on the Periphyton Community and the Water Quality of Freshwater Ecosystems.* Ecotoxicology 19 (4): 710-721. https://link.springer.com/article/10.1007/s10646-009-0446-7

Viljavuustutkimus. (2016). Eurofins Viljavuuspalvelu Oy.

Virkajärvi, P. (2003). *Effects of defoliation height on regrowth of timothy and meadow fescue in the generative and vegetative phases of growth.* Agricultural and Food Science in Finland 12: 177-193. http://urn.fi/URN:NBN:fi-fe201604069174

Wagg, C., Schlaeppi, K., Banerjee, S., Kuramae, E. E. & van der Heijden, M. G. A. (2019). *Fungal-bacterial diversity and microbiome complexity predict ecosystem functioning.* Nature Communications 10(1). doi:10.1038/s41467-019-12798-y

Wall, D. H., Nielsen, U. N. & Six, J. (2015). *Soil biodiversity and human health.* Nature 528: 69-76. doi:10.1038/nature15744

Wardle, D. A., Bardgett, R. D., Klironomos, J. N., Setälä, H., van der Putten, W. H.& Wall, D. H. (2004). *Ecological Linkages Between Aboveground and Belowground Biota.* Science 304 (5677): 1629-1633. doi:10.1126/science.1094875

Wäli, P. R., Helander, M., Nissinen, O., Lehtonen, P. & Saikkonen, K. (2008). *Endophyte infection, nutrient status of the soil and duration of snow cover influence the performance of meadow fescue in sub-arctic conditions.* Grass and Forage Science 63 (3): 324-330. doi:10.1111/j.1365-2494.2008.00639.x

Zobiole, L. H. S., Kremer, R. J., Oliveira Jnr., R. S. & Constantin, J. & Constantin, J. (2012). *Glyphosate effects on photosynthesis, nutrient accumulation, and nodulation in glyphosate-resistant soybean.* Journal of Plant Nutrition and Soil Science 175 (2): 319-330. https://doi.org/10.1002/jpln.201000434

Zobiole, L. H. S., Oliveira Jr., R. S., Kremer, R. J., Constantin, J., Bonato, C. M. & Muniz, A. S. (2010). *Water use efficiency and photosynthesis of glyphosate-resistant soybean as affected by glyphosate.* Pesticide Biochemistry and Physiology 97: 182-193. https://doi.org/10.1016/j.pestbp.2010.01.004