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Glyphosate decreases mycorrhizal colonization and affects plant-soil feedback

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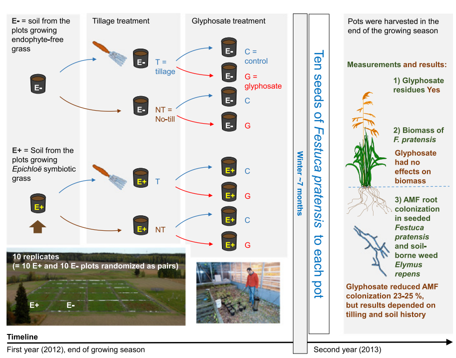
HIGHLIGHTS

- In northern ecosystems glyphosate residues are detected in crop plants the following growing season.
- Arbuscular mycorrhizal colonization is decreased in glyphosate treated plants.
- The magnitude of mycorrhizal reduction is dependent on tilling and soil history.

GRAPHICAL ABSTRACT



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ABSTRACT

Our aim was to study the effects of glyphosate, tilling practice and cultivation history on mycorrhizal colonization and growth of target (weeds) and non-target (crops) plants. Glyphosate, the world's most widely used pesticide, inhibits an enzyme found in plants but also in microbes. We examined the effects of glyphosate treatment applied in the preceding fall on growth of a perennial weed, *Elymus repens* (target plant) and a forage grass, *Festuca pratensis* (non-target plant) and their arbuscular mycorrhizal fungal (AMF) root colonization in a field pot experiment. Non-target plants were sown in the following spring. Furthermore, we tested if glyphosate effects depend on tillage or soil properties modulated by long cultivation history of endophyte symbiotic grass (E+ grass). AMF root colonization, plant establishment and growth, glyphosate residues in plants, and soil chemistry were measured. Glyphosate reduced the mycorrhizal colonization and growth of both target and non-target grasses. The magnitude of reduction depended on tillage and soil properties due to cultivation history of E+ grass. We detected glyphosate residues in weeds and crop plants in the growing season following the glyphosate treatment. Residues were higher in plants growing in no-till pots compared to conspecifics in tilled pots. These results demonstrate negative effects of glyphosate on non-target organisms in agricultural environments and grassland ecosystems.

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1. Introduction

Biocides, including pesticides, herbicides and fungicides, have been used in conventional farming practices for decades. While their use has increased crop production helping to feed globe's growing population, interest to study their risks has mainly focused on human health effects. The indirect effects of globally increasing use of biocides on non-target organisms are only rarely taken into account (Carvalho, 2016). This may translate into underestimation of the risks associated to globally increasing use of biocides to ecosystem functions and – services (Tilman et al., 2002).

Glyphosate, also known as N- (phosphonomethyl) glysine, is globally the leading herbicide of agriculture, horticulture, silviculture and urban environments in terms of both magnitude and broadness of usage (Helander et al., 2012; Myers et al., 2016). Glyphosate inactivates one part of the shikimate pathway, a metabolic route used by most plants, fungi, and bacteria for the biosynthesis of tryptophan, phenylalanine and tyrosine and molecules that require these essential proteins as precursors (Helander et al., 2012; Herman and Weaver, 1999). This pathway is not found in animal cells, and thus, glyphosate is considered to be safe for non-target organisms including vertebrates. In the connection to safe use of glyphosate against target plants, recent studies suggest possible indirect effects via soil on non-target plants, soil microbiota and microbes associated with plants and animals (Druille et al., 2013a, 2013b; Helander et al., 2012). Furthermore, the risks of the metabolites of glyphosate degradation such as AMPA [2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl) propanoic acid], surfactants and other ingredients of commercial herbicide products, have been proposed to be even more toxic than the glyphosate alone (Folmar et al., 1979; Giesy et al., 2000; Gomes et al., 2016; Tsui and Chu, 2003).

Exposure of non-target organisms to glyphosate, its degradation products and other ingredients of herbicides has exponentially increased during the third millennium (Benbrook, 2016). The patent for herbicide use of glyphosate (Monsanto Company) launched under the trade mark Roundup in 1974. Since the Monsanto's patent expired outside the USA in 1991 and in the USA in 2000, several other major manufacturers have released numerous inexpensive glyphosate-based herbicides to the market. The expanding worldwide use of glyphosate from the 1990's is for the most part a consequence of development of genetically modified, glyphosate-tolerant strains of some of the most important crop species (Benbrook, 2016; Duke and Powles, 2008; Woodburn, 2000). Glyphosate tolerant crops allow weed control of the agricultural fields after germination of the crop plants. Furthermore, glyphosate-based herbicides have enabled the no-till cropping area to increase also outside the regions where genetically modified, glyphosate resistant cultivars are used. No-till farming enables farmers to sow crops without aggressive cultivation of soil. However, this requires that weeds are decimated by multiple and timely glyphosate applications. In Northern Europe, weed control before the farming season in early spring is commonly boosted by fall glyphosate applications to control cool season perennials such as *Elymus repens.* Noteworthy is that use of glyphosate is not limited to professional use in agriculture because herbicides are readily available for non-professional users.

Although the half- time of glyphosate is shown to be within a range of days to few weeks, its occurrence in the soil may be continuous due to frequent application (Primost et al., 2017) or degradation may be prolonged due to soil properties and other environmental factors (Bai and Ogbourne, 2016). For example, glyphosate competes with phosphate for adsorption sites in soil (Gimsing and dos Santos, 2005) and its degradation is stimulated by phosphorus (Laitinen et al., 2006, 2008). It may also form complexes with metal ions (Al, Mn, Zn, Fe) (Vereecken, 2005) and attach to soil particles. High microbial activity in the soil enhances the degradation rate of glyphosate. On the other hand, glyphosate may also affect microbiota and enzymatic activities in the soil (Carlisle and Trevors, 1988; Cherni et al., 2015; Imfeld and Vuilleumier, 2012; Krzysko-Lupicka and Sudol, 2008). Thus, for example soil management practices modulating soil biotic and abiotic characteristics may alter glyphosate degradation in soil (Alvarez and Steinbach, 2009; Doran, 1980). Increasing number of field studies lends support to the idea that glyphosate inactivation and degradation in soils can be much slower than generally believed. Glyphosate and its residuals have been found to stay in the soil varying lengths of time especially in ecosystems where winters are long and cold (Helander et al., 2012). For example, as much as 19% of glyphosate and 48% of AMPA have been detected as undecomposed 20 months after application in Finland (Laitinen et al., 2009). Slow decomposition rates could partially explain the high glyphosate-related contamination levels found in Scandinavian surface waters (Ludvigsen and Lode, 2001).

Glyphosate remaining in soils can have diverse and unpredictable consequences on ecosystem functions and services (Watrud et al., 2011) especially via changes in microbial communities and their interactions with other organisms. All plants are associated with numerous microbes inhabiting both below and aboveground plant tissues. In this paper we focus on arbuscular mycorrhizal fungi (AMF) inhabiting plant roots and *Epichloë* endophytic fungi (E+) living systematically and asymptomatically in aboveground plant tissues (Saikkonen et al., 1998). Both of them are common symbionts in grassland ecosystems dominated by Pooidae grasses but functionally they differ from each other. Mycorrhizal fungi are soil-borne in contrast to *Epichloë* fungi dispersed vertically from the plant to offspring via seeds (Saikkonen et al., 2004). Both are commonly thought to be plant mutualists in many environments but benefits to host plants are different. AMF improve growth and performance of plants by increased nutrient and water uptake (Sanders and Fitter, 1992; Smith and Read, 2008) whereas the role of *Epichloë* species is multifaceted, context dependent and partly unknown (Saikkonen et al., 1998). *Epichloë* species can enhance plant growth and reproduction, and modulate chemical ecology of the symbiotum e.g. by producing herbivore- and pathogen-deterring alkaloids (Bastías et al., 2018; Hamilton et al., 2012; Saikkonen et al., 2016). By altering the amount and quality of litter, and plant exudates into the soil, *Epichloë* species associated with aboveground tissues of the host grass can affect soil biology and chemistry affecting subsequent plant performance (García-Parisi et al., 2017; García-Parisi and Omacini, 2017). Plant-soil feedback is a process through which plants alter soil biotic and abiotic properties, which then affect plant performance (Klironomos, 2002; Bauer et al., 2015). Plant root-soil feedbacks have increasingly attracted attention, but potential above-ground microbial mediated plant-soil feedback shifts have received less attention (Bastías et al., 2018; Kulmatiski et al., 2008).

Here we study if (1) glyphosate usage in the fall, (2) tillage and (3) plant-soil feedback due to cultivation history of *Epichloë* colonized grass individually or jointly affect AMF colonization and performance of target perennial weed grass (*Elymus repens*) and annually sown non-target forage grass (*Festuca pratensis*) in the next growing season. We hypothesize that, in addition to tillage, glyphosate negatively affects the perennial weed grass as the target of the fall application. Based on the observation that glyphosate and its residues can remain in soils over the winter frosts and cool summers over the years, we assume that glyphosate residues can be traced from the plants, and these residues negatively affect plant performance and AMF in the growing season subsequent to the application. Taken into account that grass symbiotic *Epichloë* endophytes may suppress AMF (García-Parisi et al., 2017), we also hypothesized that soil properties modulated by long cultivation history of *Epichloë* endophyte symbiotic (E+) grasses are unfavorable for AMF colonization in weed survivors and forage grasses.

2. Materials and methods

2.1. Soil for the experiment

The soil used in this study was collected from a long-term field experiment in Jokioinen, Finland (60° 49′ N, 23° 30′ E) in October 2012.