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Effects of glyphosate application and nitrogen fertilization on the soil and the consequences on aboveground and belowground interactions

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ABSTRACT

The application of nitrogen (N) and herbicides are commonly used to fertilize crops and protect them against weed development, but are also considered as soil and environment pollutants. Even so, the individual and combined non-target effects of N fertilizers and herbicides on multitrophic interactions within agrosystems are not well known. From soil samples collected in the field, we examined the effects of the direct application of glyphosate and/or N fertilization on microbial activities and soil nutrient status. In addition, we investigated the increase in biomass and, nutrient acquisition of the bean (*Phaseolus vulgaris*) and the consequences of the applications of N and glyphosate on the performance of the herbivore aphid (*Aphis fabae*). From soils that did (N +) or did not receive (N0) synthetic N fertilization over a 6-year period, we assessed the effects of glyphosate (CK, without glyphosate; FR, field rate of glyphosate) and N fertilization (N +, with N fertilization; N0, without N fertilization) applications in a mesocosm experiment for 75-days. Following the 75 day treatment, the biological and physiological consequences, both belowground and aboveground were determined. The growth of arbuscular mycorrhizal fungi (AMF) and dehydrogenase activity, were negatively affected following N + fertilization and the application of the FR of glyphosate, while in the absence of glyphosate, alkaline phosphatase (AIP) activity was reduced. Functional microbial responses were unaffected by both N and glyphosate, even when applied in combination. Conversely, the N fertilization significantly increased the nitrate content (NO_3^-) in the CK soils and the total N in the FR soils, compared to CK/N0 and FR/N0 soils. The combined effects of glyphosate and nitrogen fertilization (FR/N +) significantly decreased the soil C:N ratio, but significantly increased nitrification compared to CK/N0 and FR/N0 soils. The FR/N + treatments positively affected plant performance, improving the total chlorophyll, sucrose, ammonium, amino acid content, and pod biomass, compared to the CK/N0 and FR/N0 soils. Unlike glyphosate, which did not appear to exert an effect when applied alone or in combination, N fertilization significantly increased aphid nymph survival. The non-metric multidimensional scale allowed us to establish belowground and aboveground interactions with glyphosate and N fertilization. We conclude that glyphosate and N fertilization have negative effects on soil microflora and potential pests, but do not necessarily affect belowground and aboveground interactions, and may offer equal or superior benefits to crop productivity.

1. Introduction

Within a framework focused on sustainable agriculture, linking belowground and aboveground organisms in agrosystems and their response to high anthropic pressure induced by agricultural practices is a major concern. Recently, profound effects of belowground communities on aboveground insects through plant-mediated interactions have been highlighted (Bardgett and Wardle, 2010). Among the most commonly applied inputs within agrosystems are fertilizers and herbicides. Many studies report their intentional and unintended effects on trophic interactions at the aerial and soil levels within agrosystems (Birkhofer et al., 2008).

Mineral fertilizers, especially nitrogen (N), have been a major contributor to the impressive crop yield increases realized since the 1950s (Robertson and Vitousek, 2009). N may also be considered as a limiting factor for the growth of both plant and soil organisms (Mattson, 1980),

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including belowground microbial communities (Demoling et al., 2007) and the N content of a plant is one of the characteristics that is vitally important to herbivores (Mattson, 1980) in terrestrial ecosystems. In a similar manner to mineral fertilizers, pesticides may induce changes in belowground communities, modifying crop nutrient acquisition and thus relationships between plants and aboveground communities through a bottom-up effect (Saska et al., 2016).

Glyphosate (*N*-phosphonomethylglycine) is a systematic non-selective herbicide, which is the most widely used in the world to control weeds in crops (Helander et al., 2012). Glyphosate acts by inhibiting the activity of 5-*enol*pyruvylshikimate-3-phosphate synthase (EPSPS), an enzyme found in plants, bacteria and fungi (Padgette et al., 1995), some of which play key roles in soil nutrient cycling (Feng et al., 2005). Glyphosate has been shown to be rapidly decomposed by microorganisms in soil (Giesy et al., 2000) and risks of glyphosate toxicity to non-target organisms are controversial. Degradation of glyphosate depends on the composition and properties of the soil (Gimsing et al., 2007), climate conditions (Helander et al., 2012), and management practices of phosphate fertilizers (Bott et al., 2011). The primary product formed from glyphosate metabolism is aminomethylphosphonic acid (AMPA) which has great environmental persistence and mobility in soil (Kjær et al., 2005), and is also toxic to non-target organisms (Damin and Trivelin, 2011).

Soil processes related to phosphorus (P), carbon (C) and N cycles and soil quality indicators such as arbuscular mycorrhizal fungi (AMF), community-level physiological profiles (CLPP), alkaline phosphatase (AlP) and dehydrogenase (DH) activities, have been found to be very sensitive to the presence of agrochemicals (Ahtiainen et al., 2003; Goverde et al., 2000).

AMF are obligate symbionts, often working in cooperation, based on the exchange of C from the plant and P delivered by the fungi (Smith and Smith, 2011). AMF may be able to modulate the resilience of ecosystems to abiotic stresses such as nutrient deficiency, drought (Garrido et al., 2010), and biotic stresses such as plant herbivores (Shah et al., 2008; Koricheva et al., 2009). AMF are thus important ecosystem drivers (Veresoglou et al., 2012) that can improve plant growth, N uptake (Verzeaux et al., 2016), and P uptake (Goverde et al., 2000). Recent studies have shown conflicting results about the effect of glyphosate on AMF root colonization, ranging from increases to decreases or neutral effects (Malty et al., 2006; Ronco et al., 2008; Druille et al., 2013). Overall, glyphosate and N fertilization can affect soil microbial activities (such as AMF growth and colonization), which are known to create changes in foliar chemistry (increased plant P content, but reduced N) and may thus influence plant-herbivore interactions (Wurst et al., 2004).

In addition, studies have shown that interactions between belowground microbial activity and aboveground herbivore performance can occur (Tao and Hunter, 2012). Short-term changes in the nutrient quality of crop plants induced by the application of fertilizers can influence herbivore populations (Garratt et al., 2010; Tao and Hunter, 2012), and changing the ratio of macronutrients in plants can affect herbivore feeding and performance (Sterner and Elser, 2002). Indirectly, colonization by AMF positively (Gange et al., 1999) or negatively (Koricheva et al., 2009) affects herbivore performances, depending on both the herbivore and fungal species present.

A variety of carbon-containing compounds including accumulated herbicides are available for soil microorganisms, thus influencing the transformations of plant nutrients in the soil (Das et al., 2003). The community level physiological profile (CLPP) has been used in a variety of environments to assess the catabolic capacities among microbial communities (Lowit et al., 2000; Gomez et al., 2004). The responses of the CLPP and some soil enzymes can vary widely depending on the level of N fertilization. For example, several researchers have shown that high levels of N fertilization increased (Olander and Vitousek, 2000; Kalembasa and Symanowicz, 2012), or decreased (Shen et al., 2010; Kalembasa and Symanowicz, 2012) both DH and AlP activities and microbial functional diversity (Sarathchandra et al., 2001). Additionally, optimum applications of N fertilizer had a neutral effect on microbial functional activities (Lupwayi et al., 2012).

The potential perturbation of soil microbial communities and their processes has attracted interest because of the mode of action of glyphosate (Carlisle and Trevors, 1986). Glyphosate having a low C:N ratio (3:1), the excessive organic N compared to microbial demand may be readily mineralized by heterotrophic microorganisms, thus enhancing microbial activity (Haney et al., 2000). However glyphosate can be toxic for microorganisms, such as certain strains of nitrogen-fixing bacteria (Damin and Trivelin, 2011). It has been shown that glyphosate has no significant effect on soil microbial activity at the recommended field rate as compared to a 100 fold higher dose (Ratcliff et al., 2006). Moreover, glyphosate is traditionally considered to be a herbicide with relatively low ecological and toxicological side effects on terrestrial ecosystems (Giesy et al., 2000) but the impact of the application of glyphosate-based herbicides on insect herbivores, such as aphids, has been studied only rarely (Saska et al., 2016).

Inputs are often studied separately in agrosystems without considering whether they can act individually or together. In addition, studies have mainly focused on the effects of inputs either on the belowground or aboveground compartment (Barnard et al., 2006). The objective of this study was to assess the individual and combined effects of N fertilization and glyphosate applications on belowground and aboveground parameters of plants grown under laboratory conditions. Using this approach, we hypothesize that: 1) N fertilization and treatment with glyphosate can have synergistic and/or antagonistic effects on certain soil microbial activities (e.g. AMF, CLPP, soil enzymes) and the nutrient status of the soil: 2) The changes in belowground microbial activities may modulate both plant nutrient acquisition, plant biomass, and the performance of aboveground herbivores through bottom-up processes.

2. Materials and methods

2.1. Field experiment and soil sampling

Sampling was conducted in November 2015. The soil was collected from the experimental site “La Woestyne” in Northern France (50°44′N, 2°22′E, 40 m above sea level). Prior to the establishment of the experiment in 2010, the field was prepared using a chisel plough and a rotary power system, fertilized conventionally, and cultivated with wheat (*Triticum aestivum* L.). In 2010, winter cover crops (including *Vicia sativa*, *Vicia faba*, *Trifolium alexandrinum*, *Phacelia tanacetifolia*, *Avena sativa* and *Linum usitatissimum*) were sown directly, and the experimental field was split into two N fertilization regimes (without or with N fertilizer). Between 2010 and 2015, the crop rotation in each plot included green pea (*Pisum sativum* L.), maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), flax (*Linum usitatissimum* L.), beet (*Beta vulgaris* L.) and wheat. All crops were sown by using a no-till system and following a winter cover crop except wheat, which was sown directly after the maize harvest. The plot without N measured 7 × 8 m while the plot with N measured 14 × 8 m. A 7-m wide corridor separated the two plots to avoid N contamination. N fertilization in the field was determined according to the N budget method (Machet et al., 1990), and the fertilizer used consisted of 50% urea, 25% ammonia and 25% nitrate. Since 2010, a cumulative amount of 650 kg N ha^{-1} was added to the plot with N, while the plot without N was not fertilized. Since the beginning of the experiment, both plots were frequently treated with glyphosate applications for weed control. The soil is classified as a silt loam with the following properties: 66.8% silt, 21.2% clay, 12% sand. Fifty 20-cm depth soil cores were randomly sampled in each plot by using a 7-cm diameter auger. Fresh soils sampled in each plot were mixed and sieved through a 5-mm mesh. A sub-sample was then separated out, air-dried and sieved (2 mm) for chemical analyses, which were performed before the beginning of the mesocosm experiment (T0).