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Impact of glyphosate on soil microbial biomass and respiration: A meta-analysis

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ABSTRACT

The herbicide glyphosate is an important tool for weed management in many agricultural systems, but concerns have been raised that its increasing use impacts soil biology. At present, the influence of glyphosate on soil microbial biomass (SMB) and soil microbial respiration (SMR) is unclear, with inconsistent results across published studies. We hypothesised that differences in rates and formulation of herbicide application, presence or absence of plants, and variability in soil parameters such as pH and organic carbon (OC), may have contributed to the inconsistent results. To identify trends in the literature, we conducted a meta-analysis using linear mixed-effect and boosted regression tree models. Moderator variables included glyphosate concentration, soil pH, OC, planted or un-planted soils, field or pot experiments and time after glyphosate application. Glyphosate application, as well as moderator variables (pH, glyphosate concentration, OC and time after application) significantly affected microbial biomass and its activity. Increases in glyphosate and OC concentrations led to transitory enhancement (less than 60 days) of SMR and SMB, while respiration tended to be reduced after 60 days. Notably, field application rates (i.e. <10 mg kg^{-1}) had no significant effect on SMR or SMB, but SMB was significantly lower at glyphosate concentrations of 10–100 mg kg^{-1}. Ultimately, the fact that management and environmental factors regulated the soil microbial response means that generalisations about the toxicity or safety of glyphosate to SMR and SMB should be qualified with details of the conditions under which glyphosate is applied.

1. Introduction

The adoption of conservation farming techniques to improve soil structure and reduce erosion has led to an increased reliance on herbicides for weed control (D'Emden et al., 2008; Benbrook, 2012). One of the key herbicides used in conservation farming is glyphosate [N-(phosphonomethyl)-glycine], due to its low cost, its effective control of a broad spectrum of weeds and its relatively low mammalian and ecological toxicity (Baylis, 2000; Busse et al., 2001). Worldwide use of glyphosate has also increased because of the rise in cultivation of transgenic crops, of which almost 90% have glyphosate resistance (Duke and Powles, 2008).

Glyphosate prevents the growth of plants by interfering with the biosynthetic pathway of the essential aromatic amino acids needed for plant survival. It inhibits 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), a key enzyme in the shikimate pathway. Inhibition of this enzyme prevents the plant from synthesising the aromatic amino acids phenylalanine, tyrosine, and tryptophan, which are used for the synthesis of plant growth regulating compounds, cell walls, and proteins, including those involved in plant defence (Helander et al., 2012). However, the shikimate synthesis pathway is also present in microorganisms, and glyphosate may therefore also disrupt microbial growth and activity in susceptible species. Glyphosate has been shown to inhibit in vitro microbial growth of environmental isolates (Bonnet et al., 2007), food microorganisms (Clair et al., 2012) and gut microbiota at concentrations greater than 75 mg L^{-1} (Shehata et al., 2013).

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These impacts, and the increasing use of glyphosate in farming systems, have led to concerns that non-target organisms in the soil may be affected (Zabaloy et al., 2008; Helander et al., 2012). Soil microorganisms are responsible for numerous functions including nutrient cycling, soil aggregate formation and organic matter turnover; hence, impacts on the microbial community may subsequently affect soil fertility and crop production. While numerous studies have investigated the impacts of glyphosate on the soil microbiology, the results are highly variable and often contradictory. For example, glyphosate application has been reported to cause a transitory increase in soil microbial biomass (SMB) and soil microbial respiration (SMR) (Wardle and Parkinson, 1990a,b), cause significant negative impacts on microbial community structure and SMB (Andrea et al., 2003; Lancaster et al., 2009) or have no significant impact at all (Zabaloy et al., 2012; Rosenbaum et al., 2014).

It is likely that the differences in findings between individual studies are related to experimental parameters, such as differences in glyphosate application rate, properties of the soil used or indeed the environmental conditions under which the investigations are conducted. Although a review on the effects of agricultural inputs, including herbicides, on soil biology found that “the existing database [of information] is simply too small to draw sound conclusions” (Bunemann et al., 2006), a growing body of literature over the past decade may enable new insights into the impacts of glyphosate on SMB and SMR.

In recent years, meta-analyses have been increasingly used to resolve trends in soil processes from the complex and large summary data sets created through systematic literature reviews (e.g. Cayuela et al., 2014; Leifheit et al., 2014; Rose et al., 2014). These methods often provide advantages over narrative reviews by decreasing bias through sampling rigour and robust statistical methods (Arnqvist and Wooster, 1995). Quantitative data, including measured responses to experimental treatments, as well as measures of variability, are initially extracted from primary literature and categorised together with supplementary details of the experimental conditions, before being statistically analysed. The most common approach for statistical analysis is the use of linear mixed effects (LME) models, which enables variation in the data to be partitioned amongst hypothesised fixed and random effects. Unfortunately, the reliance on linear responses to factors limits the flexibility of this approach in describing non-linear relationships that commonly occur in biological and ecological systems. To overcome this, continuous variables are often delineated as categorical factors, but this can introduce bias (depending on how categorisation is made) and obscure potentially important dynamics within, or interaction between, moderators and response variables. An alternative to the use of linear mixed effects models is the application of classification and regression tree techniques. The benefit of these methods is that they do not require assumptions about the distribution of data, and they efficiently account for non-linear responses and factor interactions. They have recently been used in combination with linear mixed-effect models to explore patterns in a number of different plant—soil processes (Zhang et al., 2012; Leifheit et al., 2014; Rose et al., 2014; Zhang et al., 2015).

This study aimed to quantify the impacts of glyphosate on SMB and SMR using meta-analytical techniques to independently analyse multiple studies. We hypothesised that the inconsistencies between studies were related to differences in experimental design, including the dose and formulation of glyphosate applied; the duration of glyphosate exposure; soil characteristics such as pH and OC; the presence or absence of plants; and the use of pot or field studies. We aimed to identify the areas where risks and uncertainty exist.

2. Materials and methods

2.1. Data sources and compilation

A dataset was compiled from peer-reviewed literature by searching Scopus (Elsevier) and Web of Science (Thomson Reuters), using the keywords glyphosate, soil, (microb* OR micro-organism) and (effect OR impact) on 15th January 2015. A total of 191 unique articles were found using the keyword search and their abstracts were reviewed. The criteria for incorporation in the meta-analysis were that the study:

i) applied glyphosate or a glyphosate-containing herbicide formulation to soil;
ii) reported the rate of application in either mass of glyphosate per area of land or per mass of soil;
iii) measured at least one response variable related to SMB or SMR (as described below);
iv) used suitable controls and replication, reporting statistics or standard error.

Of the 191 papers found, 155 papers were not retained because the studies did not fit these criteria. In most cases this was because the target response variables were not measured; no glyphosate was applied as a treatment; or experiments were conducted in a soil-less medium. Data from the remaining 36 papers were extracted, resulting in a total of 558 data points addressing the impacts of glyphosate on SMB and SMR across a range of environmental and experimental scenarios.

2.2. Response variables

Numerous indicators have been proposed for assessing soil function. In order to obtain sufficient data we chose to include general indicators of SMB and SMR. These indicators are commonly measured and provide a basis for comparison between studies. The SMB response reflects the increase or decrease in the size of the microbial community. We included SMB data quantified via direct counts, chloroform fumigation extraction (Vance et al., 1987) total DNA (Marstorp et al., 2000) or total phospholipid fatty acid (PLFA) (Frostegård and Bååth, 1996). A good correlation between these different methods justifies their aggregation into a single indicator for SMB (Marstorp et al., 2000; Taylor et al., 2002; Leckie et al., 2004), especially since values are normalised as outlined below. SMR data, measured as soil CO_2 efflux, were extracted to account for the overall metabolic status of the soil biological community, being impacted by both the size of the biological community and its activity.

The impacts of glyphosate on SMB and SMR were standardised across studies by determining response ratios (R), calculated as the ratio in the response between experimental treatment mean X_e and the control treatment mean X_c, such that $R = X_e/X_c$. These ratios were log-transformed, such that $L = \ln(R) = \ln(X_e) - \ln(X_c)$, in order to overcome skewness arising from differences in numerator and denominator magnitude (Hedges et al., 1999; Johnson and Curtis, 2001). All subsequent statistical analyses (see below) used L, the ln-transformed response ratio, as the response variable.

2.3. Moderator variables

We also explored a number of factors that could influence or moderate the impact of glyphosate on the soil microbial biomass and its activity. These moderators included variables associated with glyphosate application (application rate and formulation), soil