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Changes in microbial community structure following herbicide (glyphosate) additions to forest soils

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Abstract

Glyphosate applied at the recommended field rate to a clay loam and a sandy loam forest soil resulted in few changes in microbial community structure. Total and culturable bacteria, fungal hyphal length, bacterial:fungal biomass, carbon utilization profiles (BIOLOG), and bacterial and fungal phospholipid fatty acids (PLFA) were unaffected 1, 3, 7, or 30 days after application of a commercial formulation (Roundup®). In contrast, a high concentration of glyphosate (100× field rate) simulating an undiluted chemical spill substantially altered the bacterial community in both soils. Increases in total bacteria, culturable bacteria, and bacterial:fungal biomass were rapid following application. Culturable bacteria increased from about 1% of the total population in untreated soil to as much as 25% at the high concentration by day 7, indicating enrichment of generalist bacteria. Community composition in both soils shifted from fungal dominance to an equal ratio of bacteria to fungi. Functional diversity of culturable bacteria, estimated by C substrate utilization, also increased at the high glyphosate concentration, particularly in the clay loam soil. Unlike the other bacterial indices, only minor changes in bacterial PLFA resulted after the third day following the 100× field rate application. Apparently the herbicide resulted in an across-the-board stimulation of bacteria that was not reflected by the finer-scale PLFA community structure. Changes in fungal properties (hyphae, propagules, PLFA biomarkers) were few and transient. We conclude that the commercial formulation of glyphosate has a benign affect on community structure when applied at the recommended field rate, and produces a non-specific, short-term stimulation of bacteria at a high concentration.
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Keywords: Soil bacteria; Soil fungi; Phospholipid fatty acid; Carbon utilization; BIOLOG

1. Introduction

Glyphosate is one of the most commonly used herbicides worldwide. In California alone, about 1.5 million hectares are treated annually with commercial formulations of glyphosate such as Roundup, Accord, and Rodeo (http://www.cdpr.ca.gov/docs/pur/pur-main.htm). Primarily applied to agricultural lands, glyphosate is also popular in production forestry because of its effectiveness in controlling many understory plant species, benign effects on conifers (Powers and Reynolds, 1999, 2000), low mammalian toxicity (Atkinson, 1985), and rapid inactivation in soil (Atkinson, 1985; Torstensson, 1985; Levesque and Rahe, 1992; Franz et al., 1997). However, the potential non-target effects of glyphosate on soil microorganisms and their processes, such as nutrient cycling and maintenance of soil structure, are of concern. Glyphosate inhibits protein synthesis via the shikimic acid pathway in bacteria and fungi (Bentley, 1990; Franz et al., 1997), and one of its surfactants, polyoxyethylene tallow amine, is toxic to species of bacteria and protozoa (Tsui and Chu, 2003).

Theoretical dangers notwithstanding, glyphosate has generally been found to be innocuous to soil

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microorganisms at recommended rates in field studies (Olson and Lindwall, 1991; Stratton and Stewart, 1992; Busse et al., 2001) and when applied to soil in laboratory bioassays (Stratton, 1990; Stratton and Stewart, 1992; Busse et al., 2001). High rate applications, in contrast, have been found to stimulate microbial respiration (Stratton and Stewart, 1992; Haney et al., 2000; Busse et al., 2001), and affect nutrient cycling processes (Muller et al., 1981; Carlisle and Trevors, 1986; Stratton, 1990). Glyphosate is a P-containing amino acid that functions both as a sole P source for in vitro microbial growth and as a readily available C and N source when degraded in soil (Carlisle and Trevors, 1988; Dick and Quinn, 1995; Busse et al., 2001).

Little is known of glyphosate's affect on microbial community structure (the abundance and proportion of distinct phyllogenetic or functional groups). Improvements in chemical and genetic methods of community analysis now abound (Hill et al., 2000; Sylvia et al., 2005), and provide microbial ecologists with skills to assess disturbance-caused changes in community structure (e.g. Grayston et al., 2004; Bossio et al., 2005). For example, whether the unintended consequence of glyphosate applications is to stimulate a subset of microorganisms to the detriment of other functional groups can be rigorously addressed. Popular methods include numerous DNA fingerprint techniques, PLFA, and C utilization profiles (Hill et al., 2000).

In this paper we examine changes in community structure by PLFA and C utilization analyses, supported by a coarse-level comparison of bacteria and fungi by epifluorescent microscopy and traditional culturing techniques. Our objective was to determine whether glyphosate results in short-term changes, either deleterious or beneficial, in forest soil microbial communities.

2. Materials and methods

2.1. Experimental design and field sampling

Factorial treatments including three levels of glyphosate (0, 50, and 5000 mg active ingredient kg^{-1} soil) and four sampling dates (1, 3, 7, and 30 days after application) were arranged in a completely randomized experiment. The intermediate glyphosate treatment was selected to approximate the herbicide concentration in soil after a single application at the recommended field rate of 5 kg a.i. ha^{-1} assuming (1) glyphosate movement to a depth of 1 cm in the soil profile and (2) soil bulk density of 1.0 Mg m^{-3}. The high concentration of glyphosate is essentially an undiluted application and represents a worst-case scenario following a direct chemical spill. Three replications of each treatment were included.

Soil was collected from 0 to 15 cm depth from two ponderosa pine (*Pinus ponderosa* var. *ponderosa*) plantations in northern California of differing site potential based on height growth of dominant trees at year 50 (Powers and Ferrell, 1996). The plantations, Whitmore and Elkhorn, are replicate sites of a large-scale study established to study the effects of vegetation control, fertilization, and insect control on plantation development (Powers and Ferrell, 1996). Whitmore is a low-elevation plantation located on the western slope of the southern Cascade Range (40°30′N, 121°45′W), about 40 km east of Redding, and Elkhorn is located in the Klamath Mountains (40°0′N, 122°37′W), about 50 km west of Red Bluff. The climate at both plantations is Mediterranean, with cool, moist winters and warm, dry summers (Table 1). The soil at Whitmore is formed from an andesitic mudflow and has about twice the clay and organic carbon content as the soil at Elkhorn, which is derived from uplifted metasediments (Table 1). Soil from each plantation was collected from untreated control plots (18 samples), composited, thoroughly mixed, sieved (2 mm), and stored at 4 °C until use.

2.2. Laboratory analyses

Composite soils were divided into 25 g (oven-dry equivalent) sub-samples, placed in 0.5 l jars and incubated in the dark at 20 °C for 3 days prior to herbicide application. Seventy-two samples were prepared (2 soils × 3 herbicide treatments × 4 sample dates × 3 replications). A commercial formulation of glyphosate (Roundup Weed and Grass $Killer_1$, 18%

Table 1
Soil and site characteristics of Whitmore and Elkhorn ponderosa pine plantations (from Powers and Ferrell, 1996)

	Whitmore	Elkhorn
Organic C (g kg^{-1})	50	24
Clay (%)	34	18
Silt (%)	36	38
Sand (%)	30	43
Texture	Clay loam	Loam
Soil family	Mesic Xeric Haplohumult	Mesic Typic Dystroxerept
Elevation (m)	730	1490
Precipitation (mm)	1140	1015
Site quality	Medium	Low
Year planted	1986	1988
Previous vegetation	Brushfield	Plantation

active ingredient, Monsanto Co., St. Louis, MO) was diluted to final treatment concentrations with filter-sterilized dH_2O and mixed thoroughly with soil (note: commercial trade names are for convenience only, and do not constitute endorsement by the USDA Forest Service). Samples were maintained at 60% water-holding capacity by weekly watering with filter-sterilized dH_2O. All samples were uncapped for 15 min every 2–3 days to avoid CO_2 build-up.

Soil respiration (25 g) was measured prior to subsampling for culturable bacteria and fungi, total bacteria, fungal hyphae, carbon utilization, and PLFA. Accumulated CO_2 during the previous 23 h was quantified using an infrared gas analyzer (model LI 6200, LI-COR Inc., Lincoln, NE).

Culturable bacteria and fungi were extracted by agitating a 3 g subsample of soil (oven dry equivalent) in a 10-fold dilution of 0.15 M NaCl with 3 mm glass beads for 10 min. Serial dilutions were plated in duplicate on tryptic soy agar for culturable bacteria and on malt extract agar for fungal propagules. Fungi and bacteria were counted on days 3 and 14, respectively, after growth in the dark at 28 °C. Bacterial colony types were compared visually on dilution plates containing 50–200 colonies. Dominant colony types selected from untreated and 100× field rate soils were streaked to purity and sent to Microbe Inotech Laboratories (St. Louis, MO) for identification by GC-FAME.

A second sub-sample (12 g soil, oven dry equivalent) was diluted 10-fold in 0.15 M NaCl and agitated as above. Three aliquots (10 ml each) were then taken to quantify (1) hyphal lengths (no settling), (2) total bacteria (after 5 min settling), and (3) carbon utilization (after 10 min settling).

Hyphal lengths were measured at 400× magnification using an epifluorescent microscope (filter set 01, Carl Zeiss, Inc., Thornwood, NY, USA). Extracts were diluted 20-fold (10 ml extract in 190 ml 0.15 M NaCl), then 0.5 ml aliquots were stained with fluorescent brightener 28 (2.3 g ml^{-1} dH_20; Sigma–Aldrich, St. Louis, MO, USA), incubated for 30 min, and vacuum filtered onto 0.4 μm blackened polycarbonate filters. Filters were mounted on microscope slides with immersion oil (Type A, Cargille Laboratories, Cedar Grove, NJ) placed above and below each filter. Hyphal lengths and widths were measured in 40 randomly selected fields per filter. Fungal biomass was calculated from length and width measurements using the procedure outlined by Bottomley (1994).

Total bacteria were counted at 1000× magnification (filter set 09, Carl Zeiss, Inc.). Following settling, extracts were diluted an additional 20-fold in dH_2O. Aliquots (60 ml) were filtered sequentially through 8 and 3 μm polycarbonate filters and fixed with formalin (1.8% final solution). Aliquots (2 ml) of fixed sample were stained with 20 μl acridine orange (0.01 g ml^{-1} dH_2O; Sigma–Aldrich) and incubated for 30 min. Stained samples were vacuum filtered onto 0.2 μm, blackened polycarbonate filters and mounted onto slides as above. Bacteria were enumerated on 30 randomly selected fields per filter. Total cell counts were adjusted to account for extraction losses during the initial 5 min of settling and during sequential filtering. We found clogging of the 8 and 3 μm filters by soil particles reduced total counts by 4.2- and 1.6-fold in Elkhorn and Whitmore soil, respectively. Similarly, allowing soils to settle reduced the extraction efficiency 4.6- and 3.6-fold in Elkhorn and Whitmore soil, respectively. Total counts were corrected for extraction efficiency, and bacterial biomass was calculated using the procedure of Bottomley (1994).

Carbon utilization was measured using BIOLOG GN plates (BIOLOG, Hayward, CA) by methods adapted from Garland and Mills (1991). Soil extracts were diluted an additional 15-fold in 0.15 M NaCl (final dilution = 1/150), and a 0.15 ml aliquot was added to each of the 95 wells. Preliminary experiments indicated no differences in C utilization when either sterile saline or water was used as the diluent. BIOLOG plates were incubated in the dark at 28 °C for 72 h, and metabolic potential (optical density at 590 nm) was measured three times per day. Average well color development (AWCD) of all 95 wells was corrected by subtracting the optical density of the control well. Microtiter wells with optical densities 0.2 or greater were considered positive for determining BIOLOG richness (total number of positive wells).

To determine PLFA community structure, soil lipids were extracted using a method similar to Frostegård et al. (1993). Soil (3 g) was extracted with chloroform/methanol/0.15 M citrate buffer pH 4.0 (1:1:0.8). Lipids were fractionated on silicic acid columns (J and W, Folsom, CA) into neutral, glyco-, and phospholipids as described by the manufacturer. Only the phospholipid fraction was retained for methylation. Fatty-acid methyl esters (FAME) were produced using 4% sulfuric acid in methanol (Selivonchick and Roots, 1977). Mixtures were transesterified at 95 °C for 1 h. This procedure has been shown to prevent bond migration while giving equivalent yields of FAMEs (Selivonchick and Roots, 1977).

Individual FAME were identified using a Varian 3800 gas chromatograph with a Hewlett-Packard 5973 mass-selective detector (Agilent, PaloAlto, CA) coupled to a HP-6890 engine. The capillary column was a HP-5

(30 m × 0.25 mm), with helium as the carrier gas and a temperature program as described by Frostegård et al. (1993). Total quantity (ng g^{-1} soil) of individual FAME was determined using methyl non-adecanoate as an internal standard. Phospholipid fatty acids considered of bacterial origin were 14:0, 15:0, 17:0, 18:0, i15:0, a15:0, i16:0, i17:0, a17:0, 18:1ω7, 10Me16:0, 10Me18:0, cyc17:0, and cyc19:0 (Hill et al., 2000; Myers et al., 2001). Biomarkers 18:2ω6 and 18:1ω9c were used to represent fungal PLFA (Myers et al., 2001).

2.3. Statistical analyses

Treatment effects on total and culturable bacteria, fungal hyphae and propagules, culturable:total bacteria, bacterial:fungal biomass, BIOLOG richness, and total bacterial and fungal PLFA were tested by ANOVA using the PROC GLM procedure (SAS, 2000). Normality tests (Kolmogorov–Smirnov) using the PROC UNIVARIATE procedure showed that the data for all response variables were normally distributed. Changes in individual PLFA and BIOLOG substrates due to glyphosate were tested using Tukey's studentized range test. Principal component analysis using the PROC PRINCOMP procedure and the covariance option (SAS, 2000) was used to examine PLFA and BIOLOG community structure.

3. Results

3.1. Soil respiration

Respiration was 3- to 4-fold greater following the addition of 100× field rate glyphosate compared to untreated and field rate soils. For Elkhorn soil, mean respiration plus standard error (μmol (g soil)$^{-1}$ h^{-1}) during the 30 days experiment was 0.044 ± 0.005 for untreated; 0.051 ± 0.004 for field rate and 0.155 ± 0.031 for 100× field rate. Respiration in Whitmore soil was 0.037 ± 0.005 for untreated; 0.039 ± 0.006 for field rate and 0.156 ± 0.009 for 100× field rate. The increase in respiration at 100× field rate began within 24 h of glyphosate application and was consistent throughout the experiment, resulting in significant main effects of glyphosate for both soils ($P < 0.0001$) and non-significant glyphosate × time interactions ($P > 0.2$).

3.2. Coarse-resolution community structure: microscope and plate counts

Few changes in total bacteria, culturable:total bacteria, or bacterial:fungal biomass resulted in either soil at the field rate concentration of glyphosate (Fig. 1). Fungal hyphal length and culturable bacteria were also unaffected by the field rate concentration on all sampling dates (data not shown). Fungal propagules were elevated briefly (days 1 and 3) in Whitmore soil, which resulted in a significant glyphosate × time interaction ($P = 0.02$). No significant changes in fungi were found in Elkhorn soil.

The 100× field rate concentration resulted in a shift in community structure favoring bacteria (Fig. 1). Significant glyphosate × time interactions ($P = 0.05$) were found for total bacteria, culturable bacteria, bacterial biomass, and bacteria:fungi in both soils, reflecting increases in bacterial numbers and biomass with successive sample date. No significant main effects or interactions were found for hyphal lengths or fungal biomass. As a result, the ratio of bacteria to fungi increased from ∼1:3 in untreated soils to 1:1 by day 30 in Elkhorn soil and by day 7 in Whitmore soil (Fig. 1). Interestingly, an enrichment of culturable bacteria was found in both soils. After 1 month, total bacteria in Elkhorn soil increased 4-fold while culturable bacteria increased 85-fold. In Whitmore soil, total bacteria increased 2-fold while culturable bacteria increased 11-fold. Plate counts were about 1% of total counts in untreated soils, yet were as high as 26.7% following treatment in Elkhorn soil and 5.6% in Whitmore soil (Fig. 1).

A shift in colony types was found on the dilution plates of Whitmore soil by day 30. Two colony types, preliminarily identified as *Pseudomonas putida* and *Arthrobacter protophormiae/ramosus*, accounted for 49% of all colonies in 100× field rate soil but only 5% of colonies in untreated soil. In contrast, one colony type (*Arthrobacter viscosus* or *Bacillus circulans*) was more numerous in untreated (21.3%) than 100× field rate (0%) soil by day 30.

3.3. Moderate-resolution community structure: C utilization and PLFA

A broad spectrum of BIOLOG substrates (88 out of 95 compounds) were utilized by Elkhorn soil bacteria during the first week of the experiment, with no differences between the glyphosate treatments. Thirty days after application, however, bacteria from the untreated and the field rate treatments metabolized statistically fewer substrates (82 and 79, respectively) than the high glyphosate concentration (88 substrates), resulting in a significant glyphosate × time interaction ($P = 0.003$). For Whitmore soil, mean C utilization richness during the 30 day experiment varied from 65 to 66 compounds for

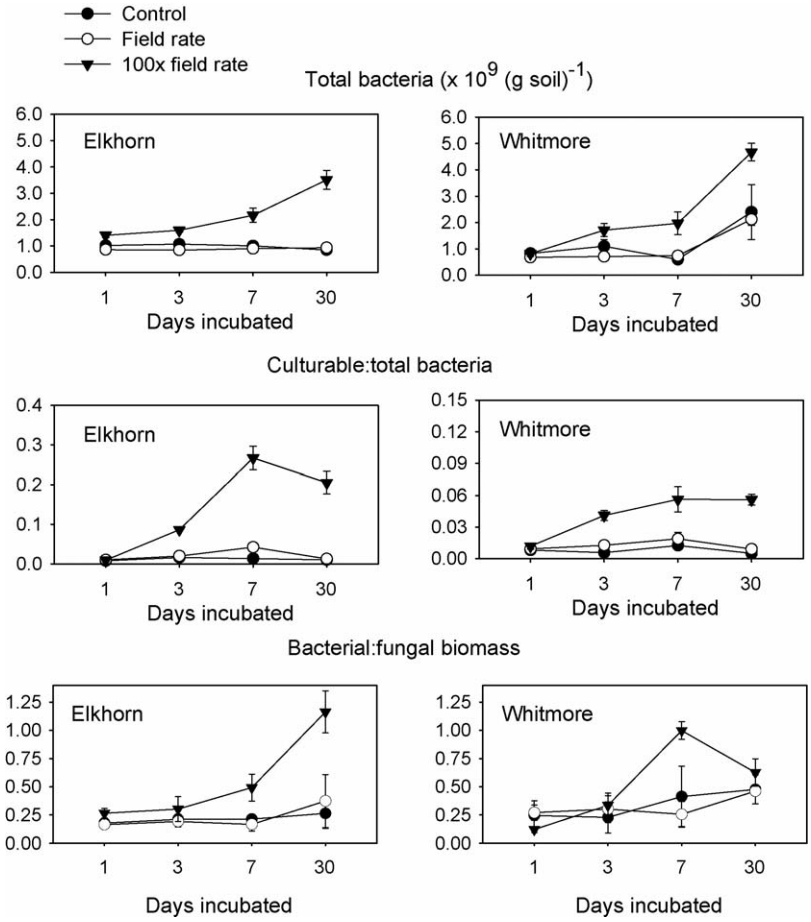

Fig. 1. Total bacteria, culturable to total bacteria, and bacterial to fungal biomass in the initial 30 days after glyphosate application to Elkhorn and Whitmore soil. Error bars represent the standard error of the mean ($n = 3$), and are not shown for values smaller than the symbol size.

untreated and field rate soils to 82 compounds for 100× soil (glyphosate main effect, $P < 0.0001$). Additional compounds metabolized by the 100× field rate treatment included several carbohydrates, carboxylic acids, and polymers. Temporal changes in C use included a slight decline in richness for untreated and field rate treatments between days 7 and 30 and an increase in richness for the 100× field rate after day 1, leading to a significant treatment × time interaction for Whitmore soil ($P < 0.0001$).

Differences in C use between the two soils was also expressed by differential rates of AWCD. Metabolic rates were similar among treatments during the first week in Elkhorn soil (Fig. 2). Only at 30 days after application was an effect of the herbicide treatments found. By comparison, strong treatment separation began on day 3 in Whitmore soil. By the end of the experiment, 55 out of 95 compounds were utilized by the 100× field rate at a statistically greater rate (Tukey's, $\alpha = 0.10$) compared to the other treatments. The metabolic rate for individual compound types (carbohydrates, carboxylic acids, amides, amino acids, polymers) followed similar patterns as AWCD.

Principal component analysis of AWCD suggests a shift in community structure for both soils 30 days after application (Fig. 3). A visual separation was found between 100× field rate and the other two treatments for both soils. The first three principal components accounted for 71% of the variation, and each component was significantly affected by the glyphosate treatment ($\alpha = 0.05$). A large, seemingly random subset of compounds was related to the treatment separation. Interestingly, a relationship appears to exist between two Elkhorn treatments (untreated, field rate) and the 100× field rate treatment in Whitmore soil, indicating a shared ability to utilize some BIOLOG substrates.

Bacterial and fungal PLFA declined during the 30 day experiment in both soils (Fig. 4). Glyphosate effects were noted in Elkhorn soil only: bacterial PLFA was lower in glyphosate-treated soil than in untreated soil on

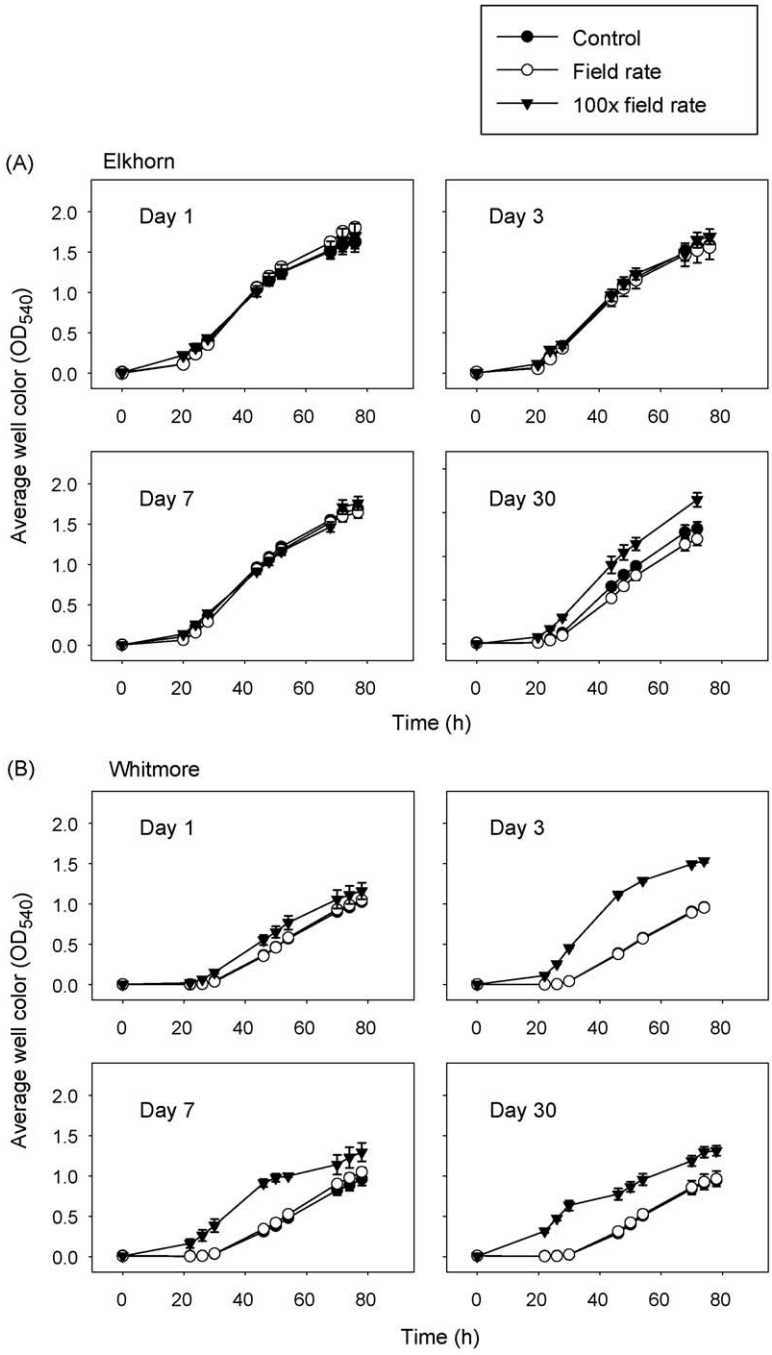

Fig. 2. Average well color development of culturable bacteria on BIOLOG GN plates following the addition of glyphosate to (A) Elkhorn and (B) Whitmore soil. Error bars represent the standard error of the mean ($n = 3$).

days 1 and 3, while fungal PLFA was lower in treated soil on day 1 only. These transient responses led to significant glyphosate × time interactions for both bacterial ($P = 0.012$) and fungal ($P = 0.003$) PLFA in Elkhorn soil. No significant main effects or interactions were found for Whitmore soil.

Despite changes in total bacterial and fungal PLFA, principal component analysis did not show any separation suggestive of a glyphosate-related shift in community structure (Fig. 5). Instead, PLFA community structure was associated with sampling date in Elkhorn soil, while no clear visual separation was found

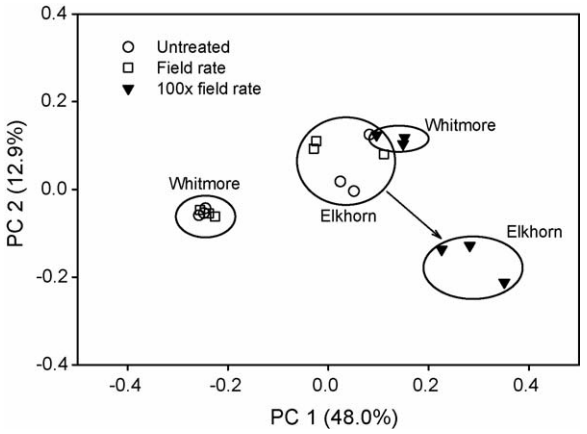

Fig. 3. Principal component analysis of BIOLOG average well color development for Whitmore and Elkhorn soil on day 30 after glyphosate application. Each symbol represents a treatment replicate ($n = 3$).

for Whitmore soil. The profiles of individual PLFA further suggest differences between sampling dates for Elkhorn soil. PLFA content was greatest and glyphosate treatment effects were found on day 1 only (Fig. 6).

4. Discussion

Stimulation of microbial activity by glyphosate has been widely reported (Grossbard, 1985; Stratton and Stewart, 1992; Haney et al., 2000; Busse et al., 2001). Glyphosate functions as an available source of P, N and C following its rapid adsorption to organic and mineral surfaces (Sprankle et al., 1975; Carlisle and Trevors, 1988; Dick and Quinn, 1995). Hence, the 30-day spike in respiration in both Elkhorn and Whitmore soil served to confirm previous observations that glyphosate is highly labile in soils (Rueppel et al., 1977; Torstensson, 1985). Of greater importance, our findings expand the current knowledge of glyphosate–soil interactions to include the non-target effect of the herbicide on microbial community structure. We found no evidence of glyphosate-induced changes in community structure at the field rate concentration. The high concentration of glyphosate, on the other hand, substantially altered several indices of community structure.

Experimental conditions in our study were selected a priori to approximate expected glyphosate concentrations for distinct soil types following a broadcast application. The field rate concentration was based on the assumption that glyphosate movement in soil is limited by chemical adsorption to the surface centimeter (Stratton, 1990), although actual penetration differs somewhat depending on soil type (Sprankle et al., 1975). Thus, community structure below 1 cm (or the majority of the mineral soil profile) is likely unaffected

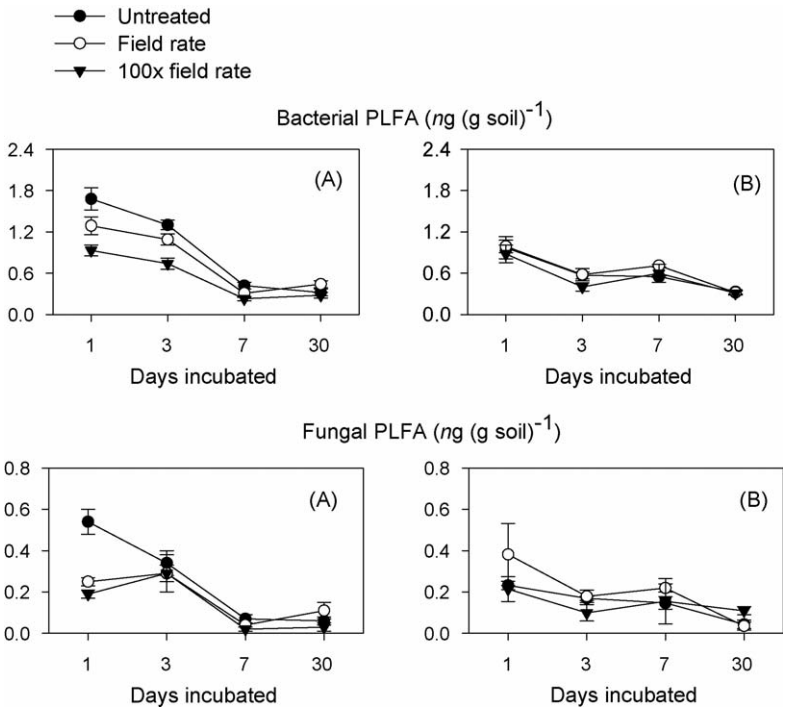

Fig. 4. Thirty-day response of bacterial and fungal PLFA to glyphosate in Elkhorn (A) and Whitmore (B) soil. Error bars represent the standard error of the mean ($n = 3$).

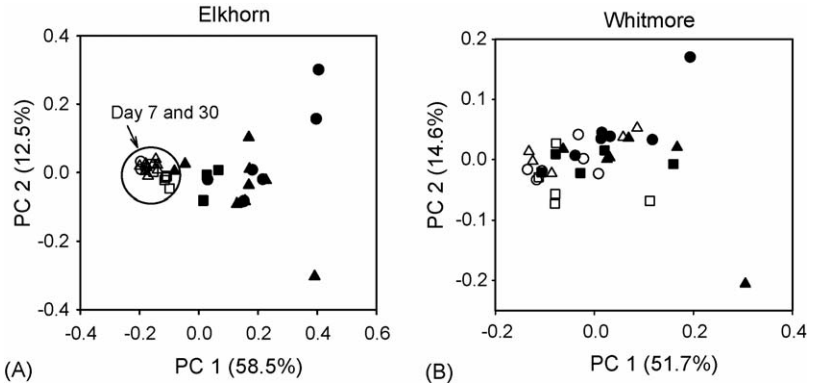

Fig. 5. (A and B) Principal component analysis of soil PLFA. Closed symbols are days 1 and 3; open symbols are days 7 and 30. Open and closed symbols: circles = untreated soil; triangles = field rate; squares = 100× field rate.

by glyphosate. Field testing is needed, however, to verify this assumption and to determine potential interactions between surface microorganisms and those found below 1 cm in the mineral soil profile. The two soils used in this study also were selected a priori based on their differing adsorptive capacities. Whitmore soil is a highly weathered Ultisol, containing considerable Fe and Al oxides, clay, and organic matter, key constituents in the adsorption of glyphosate (Sprankle et al., 1975; Torstensson, 1985; McBride and Kung, 1989; Piccolo et al., 1994). Elkhorn soil, in comparison, is a poorly developed Inceptisol, low in oxides, clay, and organic matter. Finally, we applied the commercial formulation of glyphosate which contains surfactants and additives since Busse et al. (2001) found that it had a greater affect on microbial activity than the active ingredient alone.

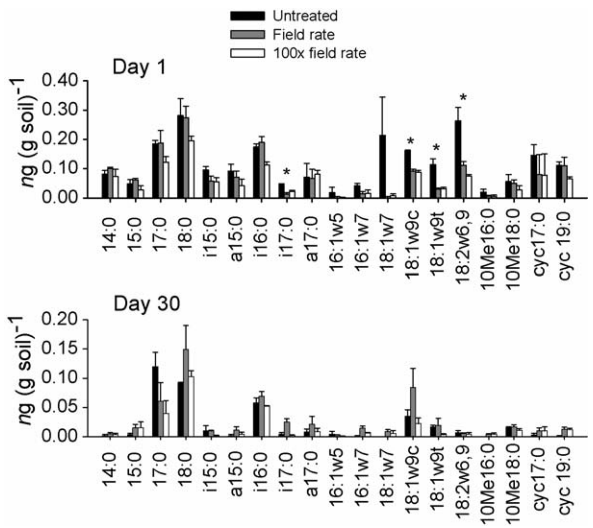

Fig. 6. PLFA profile of Elkhorn soil on days 1 and 30 following glyphosate treatment. Values are means ($n = 3$) plus standard error. *Significantly different from untreated soil by Tukey's studentized range test ($\alpha = 0.05$).

A primary effect of the high glyphosate concentration was to alter the bacterial:fungal biomass ratio. Large increases in total and culturable bacteria and bacterial biomass were found in both soils, whereas few changes in soil fungi (hyphae, propagules, biomass) resulted. This shift in community structure fits the generalized concept that bacteria are favored when labile substrates are introduced, whereas fungi are favored when complex substrates such as lignin dominate soil C (Paul and Clark, 1996; Bittman et al., 2005). Bacterial-dominated soils are most common in intensively-managed agriculture and grassland systems that rely on repeated applications of fertilizer or manure (Bardgett and McAlister, 1999; Bittman et al., 2005). It is unlikely that a single glyphosate application, even one that mimics an undiluted chemical spill, would have long lasting effects on bacterial:fungal ratios. Instead, we anticipate that the community structures in the upper profile of these two soils will return to pre-treatment conditions as the herbicide is degraded and the bacterial communities acclimate to baseline levels of labile substrates.

In conjunction with the increase in bacterial biomass, a significant enrichment of generalist bacteria, those capable of growth on complex media, was found in both soils after the 100× field rate application. Compared with untreated soil, culturable:total bacterial ratios were 6-fold greater in Whitmore soil and 25-fold greater in Elkhorn soil at the high glyphosate concentration. This finding is slightly tempered by the long-standing concern for the accuracy of plate counts and microscope counts (Richaume et al., 1993). Total microscope counts

in particular are open to criticism since they may underestimate the number of dwarf cells and fail to differentiate between living and dead cells (Klein and Paschke, 2000). Thus, culturable:total bacterial ratios effectively capture relative differences between treatments even though their absolute values may be inaccurate. In this case, the ratios clearly reflected a change in community structure favoring opportunistic, culturable bacteria. Two colony types (identified as *Pseudomonas* and *Arthrobacter*) were considerably more numerous in treated soil, perhaps not surprising given the utilitarian abilities of these genera (Hagedorn and Holt, 1975; Timmis, 2002). Since we analyzed only a limited number of colony types, however, this observation is considered preliminary.

BIOLOG results support the finding that generalist bacteria were enriched by the high glyphosate concentration. More substrates from all compound types (carbohydrates, carboxylic acids, amines, amino acids, polymers) were utilized by bacteria at the high concentration compared to untreated soil. In addition, the metabolic rate potential (AWCD) was greatest for bacteria extracted from the undiluted treatment. Again, whether this shift in the culturable community is strictly a transient response and will return to pre-treatment conditions as the herbicide dissipates or alternatively whether the addition of glyphosate resulted in an intrinsic change in community function is unclear and requires further investigation. Additionally, Bossio and Scow (1998) suggest that BIOLOG profiles may not equate with important changes in composition of the microbial community since they measure only the fastest growing portion of the community.

There were surprisingly few differences in microbial characteristics between the two soils, especially given their differences in chemical and physical properties. Whitmore is a moderately productive clay soil with relatively high organic matter content and herbicide-binding capacity. In comparison, Elkhorn represents a marginal site for plantation forestry; the soil is poorly developed, low in organic matter and fertility, and moisture limited during the growing season. Nevertheless, most of the microbial indices were comparable between soils whether glyphosate was added or not. Carbon utilization was an exception. Bacteria from Elkhorn soil utilized nearly all 95 BIOLOG compounds, whereas those from Whitmore soil utilized substantially fewer compounds. We hypothesize that bacterial acclimation to the infertile conditions at Elkhorn led to a greater zymogenous population that was capable of rapid response to labile C inputs. Close grouping of Whitmore 100× field rate and Elkhorn control samples in principal component analysis (Fig. 3) underscores the relatively large native population of generalist bacteria present in Elkhorn soil and their enrichment by glyphosate in Whitmore soil.

PLFA analysis failed to detect the shift in culturable bacteria and also did not correspond well with bacterial:fungal ratios determined by epifluorescent microscopy. Bacterial:fungal ratios increased in both soils at the high glyphosate concentration when determined by microscopy, whereas no changes were found by PLFA analysis. In fact, bacterial:fungal PLFA show a stronger response due to sample date than herbicide treatment. This finding supports previous results that show a poor relationship between PLFA and either microscope or selective antibiotic methods for determining bacterial:fungal biomass (Frostegård and Bååth, 1996; Bossio and Scow, 1998; Bailey et al., 2002; Baath and Anderson, 2003). Unlike microscopy which provides an approximation of the entire bacterial and fungal hyphal communities, PLFA quantifies selective biomarkers of bacteria and fungi and is not considered a complete assessment of the two communities (Baath and Anderson, 2003). Caution is suggested, therefore, when comparing bacterial:fungal ratios using the different techniques.

5. Summary

No major changes in microbial community structure assessed by C utilization, PLFA, and standard cultural and microscope methods were detected in forest soils following the addition of the recommended field-rate concentration of glyphosate. This finding complements previous results from an array of soils and vegetation types that glyphosate has a benign non-target effect on soil microbial activity. Addition of 100-times the field rate concentration, reflecting an undiluted chemical spill, produced a significant enrichment of bacteria and minimal change to the fungal community. As a consequence, the bacterial:fungal biomass ratio increased within 1 week of application. Culturable bacteria also comprised a greater proportion of the total population following the addition of undiluted glyphosate, and were responsible for greater C utilization of BIOLOG compounds. Since the bacterial enrichment was dose dependent, we anticipate the effects will be temporary given the rapid dissipation rate of glyphosate in soil. No evidence of detrimental change in microbial community structure due to glyphosate was found in soils with considerable differences in chemical and physical properties.

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