PERGAMON

Soil Biology & Biochemistry 33 (2001) 1777–1789

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Glyphosate toxicity and the effects of long-term vegetation control on soil microbial communities

Matt D. Busse*, Alice W. Ratcliff, Carol J. Shestak, Robert F. Powers

Pacific Southwest Research Station, USDA Forest Service, 2400 Washington Ave., Redding, CA 96001, USA

Received 6 December 2000; received in revised form 1 May 2001; accepted 21 May 2001

Abstract

We assessed the direct and indirect effect of the herbicide glyphosate on soil microbial communities from ponderosa pine (*Pinus ponderosa*) plantations of varying site quality. Direct, toxic effects were tested using culture media and soil bioassays at glyphosate concentrations up to 100-fold greater than expected following a single field application. Indirect effects on microbial biomass, respiration, and metabolic diversity (Biolog and catabolic response profile) were compared seasonally after 9–13 years of vegetation control using repeated glyphosate applications in a replicated field study. Three pine plantations were selected to provide a range of soil characteristics associated with glyphosate binding (clay, Fe and Al oxide content) and site growing potential from the lowest to the highest in northern California. Glyphosate was toxic to bacteria and fungi from each plantation when grown in soil-free media. Culturable populations were reduced, as was the growth rate and metabolic diversity of surviving bacteria, by increasing concentrations of glyphosate. This toxicity was not expressed when glyphosate was added directly to soil, however. Microbial respiration was unchanged at expected field concentrations (5–50 $\mu g\ g^{-1}$), regardless of soil, and was stimulated by concentrations up to 100-fold greater. Increased microbial activity resulted from utilization of glyphosate as an available carbon substrate. Estimated N and P inputs from glyphosate were inconsequential to microbial activity. Long-term, repeated applications of glyphosate had minimal affect on seasonal microbial characteristics despite substantial changes in vegetation composition and growth. Instead, variation in microbial characteristics was a function of time of year and site quality. Community size, activity, and metabolic diversity generally were greatest in the spring and increased as site quality improved, regardless of herbicide treatment. Our findings suggest that artificial media assays are of limited relevance in predicting glyphosate toxicity to soil organisms and that field rate applications of glyphosate should have little or no affect on soil microbial communities in ponderosa pine plantations. Published by Elsevier Science Ltd.

Keywords: Ponderosa pine; Roundup; Microbial biomass; Substrate-induced respiration; Metabolic diversity

1. Introduction

Glyphosate is a widely popular herbicide known for its effective control of competing vegetation, rapid inactivation in soil, and low mammalian toxicity (Levesque and Rahe, 1992; Franz et al., 1997). Although primarily used on agricultural lands and rights-of-way, glyphosate is often the preferred herbicide in intensive forestry due to its mild effect on conifers. About 40,000 ha of forest land are treated annually with glyphosate in California alone (Calif. Dept. Pesticide Regulation, 2000). Improvements following glyphosate application range from plantation survival on harsh sites to large increases in stand productivity on average and better sites (Powers and Reynolds, 1999, 2000).

Unwanted side effects on non-target organisms are an environmental concern with the use of xenobiotic compounds. In the case of glyphosate, potential disruption of soil microbial communities and their processes has attracted interest because of the compound's mode of action (Carlisle and Trevors, 1988). Glyphosate inhibits 5-enolpyruvylshikimic acid-3-phosphate synthase, an intermediate enzyme in aromatic amino acid synthesis via the shikimic acid pathway (Franz et al., 1997). Most living organisms, excluding plants, lack this pathway and are thus unaffected directly by glyphosate. The shikimic acid pathway is ubiquitous in microorganisms, however (Bentley, 1990). Reports of harmful effects to microorganisms are numerous in laboratory studies (Christy et al., 1981; Quinn et al., 1988; Santos and Flores, 1995; Krzysko-Lupicka and Orlik, 1997). Microbial growth in artificial media containing glyphosate as the sole C or N source is rare, and only a limited number of bacterial and fungal species are capable of growth when

* Corresponding author. Tel.: +1-530-242-2456; fax: +1-530-242-2460.
E-mail address: mbusse@fs.fed.us (M.D. Busse).

0038-0717/01/$ - see front matter Published by Elsevier Science Ltd.
PII: S0038-0717(01)00103-1

glyphosate is supplied as the sole P source (Liu et al., 1991; Dick and Quinn, 1995; Krzysko-Lupicka and Orlik, 1997).

Contrary to laboratory results, most agricultural field studies have shown either no affect or a slight stimulation of soil microorganisms by glyphosate. Increases in culturable bacteria and fungi (Roslycky, 1982; Rueppel et al., 1977), soil respiration (Carlisle and Trevors, 1986a; Haney et al., 2000), N mineralization (Haney et al., 2000), and soil enzyme activity (Gianfreda et al., 1995) are reported, while negligible changes in N cycling processes (Carlisle and Trevors, 1986b; Olson and Lindwall, 1991; Muller et al., 1981) and microbial biomass (Wardle and Parkinson, 1991, 1992) have also been observed. Discrepancies between laboratory and field studies can be explained partially by unrealistically high herbicide concentrations used in many laboratory studies (Wardle, 1995) and by herbicide chemistry. Glyphosate is a polar compound known for its strong adsorption to Fe and Al oxides and clay (McBride and Kung, 1989; Piccolo et al., 1994; Morillo et al., 1997). Although its availability is unrestricted in artificial media, binding to soil particles and metal complexes reduces the pool of labile glyphosate and, consequently, the uptake rate by soil microbes. This raises the question of whether microbial communities in soils with low adsorption capacity are at greater risk following spraying due to high concentrations of labile glyphosate. Evidence to support this possibility has been inconclusive to date (Rueppel et al., 1977; Gianfreda et al., 1995; Eberbach, 1998).

Most information on the non-target effects of glyphosate comes from agricultural studies. Knowledge of forest soil microorganisms and their response to glyphosate is limited and somewhat contradictory. For example, Stratton and Steward (1992) found no change in microbial biomass in mineral soil and a stimulation of microbial biomass in litter following addition of glyphosate to an acidic forest soil. Mycorrhizal infection of red pine (*Pinus resinosa*) was also unaffected by glyphosate application (Chakravarty and Chatarpaul, 1990). In contrast, detrimental effects of glyphosate to total soil C and N pools were found in a northern boreal forest (Munson et al., 1993), and alteration of N immobilization and nitrate leaching was found in a southern pine plantation (Vitousek et al., 1992). Long-term, continuous vegetation control by a combination of herbicide applications and hand grubbing has also been shown to reduce soil microbial biomass and total C in the upper surface of young ponderosa pine stands (Busse et al., 1996).

We capitalized on the 'Garden of Eden' experiment, a long-term field study of intensive management of ponderosa pine plantations (Powers and Ferrell, 1996), to evaluate the non-target effects of glyphosate on soil organisms. Treatments included up to 13 years of repeated understory vegetation control using annual applications of glyphosate, replicated at three sites of differing soil type in northern California. Our objectives were to determine (1) whether glyphosate results in direct (toxic) or indirect, long-term changes in microbial biomass, activity, and metabolic diversity in pine plantations; (2) whether microbial responses to glyphosate vary with soil type, site quality, or time of year; (3) the appropriateness of artificial media studies in predicting environmental responses to glyphosate.

2. Materials and methods

2.1. Study sites and treatment design

The three plantations chosen for study (Elkhorn, Whitmore, Feather Falls) were located in separate geomorphic provinces in northern California. Elkhorn is located about 50 km west of Red Bluff at an elevation of 1490 m in the Klamath Mountains; Whitmore is located about 50 km east of Redding at an elevation of 730 m in the southern Cascade Range; and Feather Falls is located about 35 km east of Oroville at an elevation of 1220 m in the northern Sierra Nevada Mountains.

Selection of the study sites was based on providing a range of (1) site potential for growing ponderosa pine, (2) glyphosate-adsorption potential in the surface mineral soil as predicted by clay and oxide content. Elkhorn is the poorest growing site among the three plantations (site index of 17 m on a 50-year basis (Powers and Oliver, 1978)). Vegetation growth is limited by moisture availability (1020 mm annual precipitation) and infertile, skeletal soil. The soil (loamy-skeletal, mixed, active, mesic Typic Dystroxerept) has low glyphosate adsorption potential, with minimal oxide content, clay content of 18%, organic matter content of 20.5 g kg^{-1}, and pH of 5.9 in the surface 0–10 cm. Whitmore is an intermediate growing site (site index of 23 m), limited by hot (mean daily temp in August is 25.4°C), dry (1140 mm annual precipitation) summer climate. The soil (clayey, mesic Xeric Haplohumult) has high potential for glyphosate adsorption, with high oxide content, clay content of 34%, organic matter content of 39.1 g kg^{-1}, and pH of 5.6. Feather Falls is among the most productive sites in northern California (site index >30 m) owing to its mild and relatively moist (1780 mm) climate. The soil (loamy, mesic Ultic Haplxeralf) has an intermediate glyphosate-binding potential, with moderately-high oxide content, clay content of 28%, organic matter content of 66.9 g kg^{-1}, and pH of 5.4.

The study sites were planted with 1-year-old ponderosa pine seedlings at a 2.5 m square spacing. Elkhorn was planted in 1988 following clearing of a poorly-stocked, brush-choked pine plantation. Whitmore was planted in 1986 after brush-rake clearing of a brushfield of whiteleaf manzanita (*Arctostaphylos viscida*) and common manzanita (*Arctostaphylos manzanita*). Feather Falls was planted in 1988 after harvesting of a natural stand of conifers and hardwoods and brush-raking of residues.

The plantations are part of the Garden of Eden study, which tests the effect of competing vegetation, nutrient

limitations, and insect damage on ponderosa pine growth (Powers and Ferrell, 1996). Treatments include factorial combinations of herbicide, fertilizer, and insecticide, and are replicated three times at each of eight plantations. Plot size is 0.04 ha and the experimental design is a randomized complete block design. A subset of treatments was selected for our purposes: (1) herbicide, and (2) control (no treatment). The herbicide treatment received repeated applications of a commercial formulation of glyphosate (Roundup). Glyphosate was manually applied from a backpack sprayer at the recommended field concentration (3 kg a.i. ha^{-1}) from 1986 to 1995 at Whitmore, from 1988 to 1995 at Elkhorn, and from 1988 to 1996 at Feather Falls. Sparse (or no) understory vegetation survived in the 2–3 years following the final herbicide application. Understory vegetation on control plots was shrub dominated, with cover of 25% at Elkhorn, 94% at Whitmore, and 110% at Feather Falls after 10 years (Powers and Reynolds, 1999).

2.2. Direct effects on culturable organisms and total soil respiration

Mineral soil (0–10 cm depth) was collected from replicate plots of the control treatment at each site to determine culturable population size, bacterial growth rate, and total soil respiration response to glyphosate addition. Each sample was a composite, pooled from eight randomly-collected subsamples per plot. Samples were sieved (2 mm) and stored at 4°C until 72 h prior to analysis, when they were wetted to approximately 80% of field capacity and incubated at 22°C. Ten-fold serial dilutions in 0.15 M NaCl were plated in duplicate on tryptic soy agar (TSA) containing 0, 25, 50, or 500 mM filter-sterilized glyphosate and on malt extract agar containing 0 or 50 mM filter-sterilized glyphosate. Glyphosate additions to TSA represent 0-, 0.5-, 1.0-, and 10-times, respectively, the concentration recommended for a tank-mixture application. Total culturable bacteria were counted on day 14 and spore-forming fungi on day 3.

Bacteria were extracted from the composite samples and their whole-community growth rate was determined by the average well color development on Biolog GN plates (Garland and Mills, 1991). Soil (3 g) was diluted in 27 ml of 0.15 M NaCl and mixed for 10 min on an orbital shaker. After settling (10 min), 10 ml of supernatant was added to 140 ml solutions containing 0, 25, 50, or 500 mM filter-sterilized glyphosate in sterile saline. A final volume of 0.15 ml was added to each microtiter-plate well. Plates were read for 10 days, during which no contamination of the control wells (water only) was found.

Soil respiration was measured in laboratory bioassays for 10 days after the addition of glyphosate as active ingredient (0, 5, 50, 500 mg kg^{-1}) or commercial formulation (0, 5, 50, 500, 5000 mg a.i. kg^{-1}) to each sample. Five and 50 mg kg^{-1} were considered the lower and upper limits, respectively, of the expected glyphosate concentration in soil assuming (i) 5 kg a.i. applied ha^{-1}, as suggested by the manufacturer, (ii) herbicide movement between 1 and 10 cm in the soil profile, (iii) soil bulk density of 1.0 Mg m^{-3}. Twenty-five g samples were wetted to approximately 80% of field capacity and incubated at 22°C in 500 ml mason jars with fitted septa. After 72 h, glyphosate solutions (2 ml) were added to each soil, thoroughly mixed, and daily CO_2 concentrations were determined using an infra-red gas analyzer (LiCor 6200). Mason jars were uncapped for 15 min following each reading to approximate ambient CO_2.

2.3. Indirect effects of continuous vegetation control

Mineral soil (0–10 cm depth) was collected seasonally in 1998 from each replicate of the herbicide and control treatments at the three sites. Each sample (a composite of eight randomly-collected subsamples) was sieved (2 mm) and analyzed within 24 h of collection for gravimetric moisture content, microbial biomass by substrate-induced respiration (Anderson and Domsch, 1978), basal respiration (Zibilske, 1994), total bacteria by acridine orange direct count (Bottomley, 1994), and metabolic diversity of culturable bacteria (Garland and Mills, 1991) and whole community (autumn samples only; Degens and Harris, 1997). Seasonal collection dates were 6/8, 8/17, 10/19 at Elkhorn; 5/18, 7/27, 11/13 at Whitmore; and 6/1, 8/3, 10/2 at Feather Falls. Samples from Whitmore (5/18) and Feather Falls (6/1) were too wet to effectively sieve. Instead, large soil particles and roots were removed by hand sorting. Organic matter content, determined by loss-on-ignition (Nelson and Sommers, 1982), and mineralizable N (Powers, 1980) were measured using spring samples only. Total soil C was estimated by dividing organic matter content by 1.72.

For total bacteria, a minimum of 10 fields were counted per filter. Bacteria were counted separately by size class (<0.4, 0.4–2.0, >2.0 μm) and their biomass was calculated by the method outlined by Bottomley (1994), using a dry weight/biovolume conversion factor of 0.33 g cm^{-3}.

Metabolic diversity of culturable bacteria was measured using Biolog GN plates. Inoculum densities were not standardized prior to inoculation of plates, although concurrent measurement of total bacteria showed no differences between samples for each site and collection date. Optical density (590 nm) readings were taken a minimum of 12 times during the initial 72 h, and average well-color development was calculated by the integral of each growth curve (Guckert et al., 1996) in order to account for community lag time and specific growth rate.

Metabolic diversity of the total community was measured by the catabolic response profile method outlined by Degens and Harris (1997). Briefly, this method compares substrate-induced respiration (SIR) patterns following the addition of individual C compounds. Thirty-six compounds (Table 1) were selected based on their predicted ability to discriminate between herbicide and control treatments. Specific

Table 1
Sole carbon sources used to measure catabolic response profiles

Carbohydrates	*Carboxylic acids*
α-D-glucose	Acetic acid
α-D-lactose	Formic acid
Turranose	D-galacturonic acid
Adonitol	γ-hydroxybutyric acid
i-erythritol	Itaconic acid
D-mannitol	α-ketoglutaric acid
β-methyl-D-glucoside	D, L-lactic acid
D- L-glycerol phosphate	Propionic acid
Glucose-1-phosphate	Succinic acid
Amino acids	*Polymers*
D, L-alanine	α-cyclodextrin
L-asparagine	Glycogen
Gly-glu	Tween 40
L-ornithine	Tween 80
L-phenylalanine	
L-proline	*Nucleosides*
D-serine	Thymidine
L-serine	Uridine
L-threonine	
Amines	*Amides*
N-acetyl-D-glucosamine	Glucuronamide
Putresine	

criteria included the selection of compounds that either (1) explained most of the variability in principle component analysis of Biolog data from spring and summer samples, (2) were responsible for treatment separation in Biolog tests, or (3) were recommended by Insam (1997). Duplicate, 2 g (oven-dry equivalent) samples from each replicate plot of herbicide and control treatments were incubated in the dark at 22°C in 20 ml vials for 72 h prior to addition of single C compounds. Soil moisture contents were adjusted to field capacity immediately following the addition of the C compounds. Respiration was then measured within 6 h using an infra-red gas analyzer. Carbon utilization of each compound was considered the proportional increase in SIR relative to basal respiration (soil + H_2O only).

2.4. Carbon, N, and P addition experiment

The contribution of glyphosate-derived C, N, and P to microbial activity was tested in a nutrient addition experiment. Factorial combinations of C (0, 0.54 g glucose kg^{-1}), N (0, 0.24 g NH_4NO_3 kg^{-1}), and P (0, 0.80 g KH_2PO_4 kg^{-1}) were added to 25 g of soil collected from the control plots at Whitmore, and respiration rates were compared with samples receiving the highest concentration of glyphosate (5.0 g kg^{-1}). All samples were wetted to approximately 80% of field capacity. Three replicates of nine treatments (eight factorial treatments plus the glyphosate-treated soil) were included, and respiration was measured every 24 h for 5 days. Our intent was to add C, N, and P in proportion to their mole fraction in the commercial formulation of glyphosate. However, this information is not shared by the manufacturer. Selection of C, N, and P concentrations, instead, was based on preliminary, concentration-gradient experiments that identified the minimum concentration of each compound (within a range from 1 to 80% mole fraction of the commercial formulation) that provided optimum stimulation of soil respiration.

2.5. Statistical analyses

Repeated measures analysis using the PROC MIXED procedure (SAS, 1995) was used to test the long-term effect of glyphosate on microbial biomass, respiration, total bacteria, metabolic quotient (biomass/respiration), and the ratio of microbial C to total C (Cm:Ct). No departures from normality were observed for the residuals of any of the models. Metabolic diversity of culturable bacteria was compared using principal component analysis (SAS, 1995). The C, N, and P addition experiment was analyzed by ANOVA and Tukey's honestly significant difference test for separation of means. Differences between treatments were considered significant at $P < 0.05$.

3. Results

3.1. Direct effects of glyphosate on viable growth and soil respiration

Addition of glyphosate to culture media resulted in a reduction in culturable bacteria and spore-forming fungi (Table 2). Fungi were particularly sensitive. No fungal growth was detected for the Elkhorn and Whitmore soils at the recommended sprayer concentration of 50 mM. Bacteria from Elkhorn and Whitmore samples were below the limit of detection (10^3 CFU g^{-1}) at 500 mM. Differences between sites were minor. Bacteria and fungi extracted from Feather Falls soils showed slightly greater tolerance to glyphosate than organisms from either Elkhorn or Whitmore.

Bacteria were grown in Biolog GN media for 10 days to

Table 2
Reduction in culturable bacteria and fungi on media containing glyphosate. Organisms were plated on tryptic soy agar (bacteria) and malt extract agar (fungi). Recommended sprayer application concentration is approximately 50 mM glyphosate. Means plus standard deviation in parentheses ($n = 3$) are given. No growth detected at the lowest dilution (10^3) is indicated by NG

Glyphosate concentration	Elkhorn	Whitmore	Feather Falls
Bacteria (CFU × 10^6 g^{-1})			
0 mM	12.3 (1.2)	7.7 (1.1)	17.0 (3.6)
25 mM	0.6 (0.2)	0.2 (0.1)	0.7 (0.7)
50 mM	0.2 (0.1)	0.1 (0.1)	0.4 (0.4)
500 mM	NG	NG	0.03 (0.04)
Fungi (CFU × 10^5 g^{-1})			
0 mM	4.1 (0.6)	4.0 (2.7)	60 (35)
50 mM	NG	NG	0.2 (0.1)

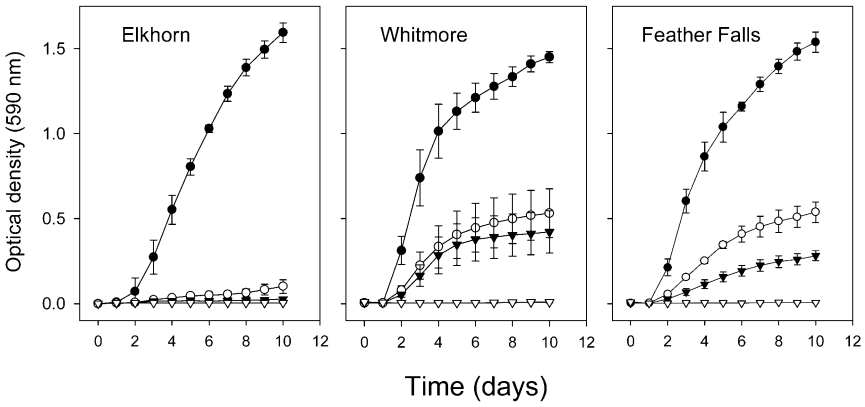

Fig. 1. Bacterial growth rate on Biolog GN plates following addition of 0 mM (●), 25 mM (○), 50 mM (▼), or 500 mM (▽) glyphosate. Recommended sprayer application concentration is approximately 50 mM glyphosate. Error bars are ±1 standard deviation.

determine the growth rate and metabolic diversity of organisms that tolerate glyphosate. Addition of 25 and 50 mM glyphosate reduced the growth rate of bacteria from each plantation soil (Fig. 1). The effect was strongest on bacteria from Elkhorn, although no growth was detected at 500 mM glyphosate in any soil. Metabolic diversity of culturable bacteria also declined rapidly due to glyphosate. Species richness (no. C compounds metabolized) at 0, 25, 50 and 500 mM glyphosate was 39, 1, 0, 0 for Elkhorn; 60, 34, 29, 0 for Whitmore; and 62, 28, 6, 0 for Feather Falls, respectively.

Glyphosate had no measured effect on soil respiration when added to soil at normal field concentrations and stimulated respiration at high concentrations (Fig. 2). Soil respiration following the addition of 5 and 50 mg kg^{-1} glyphosate was equal to the control soil from each plantation. This is the expected range in concentration following broadcast application of glyphosate, assuming the herbicide moves to a depth of 1–10 cm in the soil profile. Similar results were found when the active ingredient was added to each soil (data not shown).

3.2. Indirect effects of repeated glyphosate additions

Microbial characteristics in the surface 10 cm of soil were generally unchanged after 9–13 years of continuous

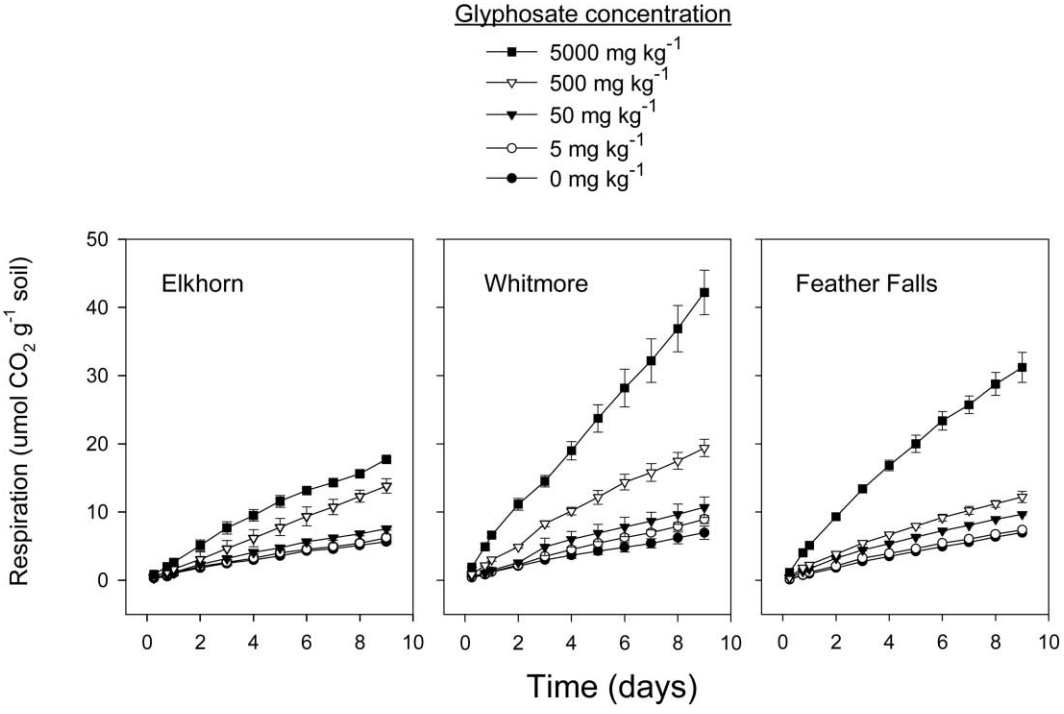

Fig. 2. Cumulative soil respiration following addition of glyphosate. Estimated field concentration is 5–50 mg kg^{-1} assuming herbicide movement between 1 and 10 cm in the soil profile. Error bars are ±1 standard deviation.

vegetation control by glyphosate. There was no effect of glyphosate on basal respiration, metabolic quotient, or total bacteria (Tables 3 and 4). Mineralizable N, organic matter content, and seasonal gravimetric moisture content were also unaffected by glyphosate. Microbial biomass and the ratio of microbial C to total C (Cm:Ct) showed inconsistent seasonal declines following repeated herbicide treatment. Mean microbial biomass between spring and autumn sampling declined 20% at Elkhorn, 7% at Whitmore, and increased 5% at Feather Falls due to continuous vegetation control (Table 3), resulting in a significant time × herbicide interaction in repeated measures analysis (Table 4). Effects of glyphosate on Cm:Ct were strongest at Elkhorn, and resulted in a significant site × herbicide interaction.

Glyphosate effects were minor compared to the main effects of site and sampling date which influenced all measured indices of microbial population size and activity. For example, microbial biomass mirrored the site quality gradient. Feather Falls, the most productive site, had the highest microbial biomass at each sample date, whereas Elkhorn, the least productive site, consistently had the lowest microbial biomass. Seasonal patterns of microbial biomass varied by site. A progressive decline in microbial biomass was found at Whitmore and Feather Falls during the growing season, whereas peak biomass was found during the summer at Elkhorn. As a result, a significant site × time interaction was found (Table 4).

Minimal change in the metabolic diversity of culturable bacteria was found after long-term vegetation control. Average well color development was similar between glyphosate and control treatments at each plantation and sampling date (Fig. 3). Response curves for each compound type (carbohydrate, carboxylic acid, amino acid, amine, amide, and polymer) had similar growth patterns and lack of treatment separation (data not shown). The only exception was the autumn sample at Whitmore which had greater C utilization for all compound types by the control compared to the glyphosate treatment. Reasons for this are unclear, although two potential sources of variation, soil moisture and inoculum size, can be ruled out. Soil moisture content was nearly identical between treatments (26.7% for glyphosate and 26.8% for control treatment), and the inoculum size of total bacteria was only slightly greater for the control treatment (Table 3).

A strong seasonal trend in C utilization was found. Carbon utilization was greatest in spring, and dropped precipitously in the summer, particularly at Elkhorn and Whitmore. Growth rates failed to recover at Elkhorn by the autumn, likely due to the lack of precipitation in the autumn of 1998. Soil moisture content in the autumn was only 4.5% at Elkhorn compared to 26.7% at Whitmore and 39.2% at Feather Falls. No differences between herbicide and control treatments were found in substrate richness (no. positive wells) or diversity (Shannon Weaver Index) between herbicide and control treatments at any site or sampling date with the exception of the Whitmore autumn sample.

Principal component analysis identified a strong seasonal trend in the metabolic diversity of culturable bacteria. At Elkhorn, spring samples were strongly separated from summer and autumn samples (Fig. 4). This followed the pattern of soil moisture content: moist in spring and dry in summer and autumn. No differences between treatments were evident. Metabolic diversity at Feather Falls was comparable between spring and autumn samples, indicating similar recovery by glyphosate and control treatments after the dry summer. Recovery at Whitmore varied with treatment. Metabolic diversity of the glyphosate treatment was comparable between spring and autumn samples, while the control treatment showed separation between the two sample dates.

Catabolic response profiles were compared as an alternative method of testing metabolic diversity. Differences in SIR between treatments were generally small, and the trends were inconsistent among types of C compounds and sites (Table 5). Out of 36 compounds, only 3 (α-D-lactose, adonitol, L-proline) showed significant treatment effect at Elkhorn, 2 (*i*-erythritol, formic acid) at Whitmore, and 1 (α-D-glucose) at Feather Falls. This is further indication of limited differences between microbial communities after long-term vegetation control treatment.

3.3. Carbon, N, P addition experiment

Combinations of available C, N, and P were added to the Whitmore soil to determine if the stimulation of soil respiration by high concentrations of glyphosate was the result of energy and/or nutrient input from the herbicide. Only those treatments that received available carbon showed a positive response in respiration (Table 6). Combinations of N and P additions had no affect, indicating the stimulation of microbial activity at high glyphosate concentrations was a result of C utilization.

4. Discussion

Our interest in glyphosate and its affect on soil microorganisms was kindled by the putative toxicity of this popular herbicide to microorganisms and by evidence that showed a decrease in microbial biomass after long-term control of competing vegetation (Busse et al., 1996). Several standard indices of microbial community size, activity, and metabolic diversity were tested for their response to glyphosate. These included biomass (substrate-induced respiration), basal respiration, plate counts, total counts, metabolic diversity (Biolog and catabolic response profile), and mineralizable N. Laboratory and field experiments were designed for comparison of both herbicide toxicity and indirect changes due to continuous vegetation control. All experiments included comparisons of soils of diverse clay, oxide, and organic matter content from plantations ranging in productivity

Table 3
Seasonal microbial characteristics in 1998 following long-term, repeated applications of glyphosate at plantations of low (Elkhorn), medium (Whitmore), and high (Feather Falls) site productivity. Means plus standard deviation in parentheses ($n = 3$) are given

Variable	Spring		Summer		Autumn	
	Glyphosate	Control	Glyphosate	Control	Glyphosate	Control
Microbial biomass ($\mu g\ g^{-1}$)						
Elkhorn	227 (52)	325 (83)	396 (76)	507 (52)	320 (38)	350 (50)
Whitmore	548 (98)	497 (47)	444 (3)	478 (98)	425 (35)	541 (39)
Feather Falls	1366 (159)	1093 (236)	665 (67)	673 (94)	505 (78)	644 (177)
Basal respiration ($\mu mol\ g^{-1}\ h^{-1}$)						
Elkhorn	0.16 (0.05)	0.21 (0.03)	0.13 (0.07)	0.10 (0.02)	0.07 (0.02)	0.09 (0.02)
Whitmore	0.27 (0.07)	0.23 (0.02)	0.13 (0.04)	0.13 (0.02)	0.15 (0.02)	0.18 (0.01)
Feather Falls	0.45 (0.07)	0.43 (0.05)	0.08 (0.01)	0.06 (0.03)	0.18 (0.03)	0.23 (0.08)
Metabolic quotient (resp $biomass^{-1}$)						
Elkhorn	0.70 (0.03)	0.64 (0.08)	0.31 (0.12)	0.20 (0.04)	0.20 (0.04)	0.26 (0.03)
Whitmore	0.48 (0.13)	0.44 (0.09)	0.29 (0.09)	0.27 (0.02)	0.36 (0.05)	0.34 (0.02)
Feather Falls	0.33 (0.07)	0.41 (0.10)	0.11 (0.01)	0.09 (0.04)	0.35 (0.02)	0.37 (0.13)
Cm:Ct[a] ($g\ g^{-1}$)						
Elkhorn	1.1 (0.3)	1.8 (0.6)	1.9 (0.2)	2.7 (0.1)	1.5 (0.3)	1.9 (0.2)
Whitmore	1.4 (0.3)	1.4 (0.2)	1.0 (0.1)	1.2 (0.3)	1.0 (0.1)	1.4 (0.2)
Feather Falls	1.9 (0.2)	1.6 (0.2)	0.9 (0.1)	1.0 (0.2)	0.7 (0.1)	0.9 (0.2)
Total bacteria ($\times 10^8\ g^{-1}$)						
Elkhorn	0.3 (0.1)	0.3 (0.0)	0.3 (0.1)	0.3 (0.0)	nd	nd
Whitmore	2.0 (0.4)	2.0 (0.4)	1.0 (0.1)	1.1 (0.1)	1.7 (0.1)	1.9 (0.3)
Feather Falls	0.9 (0.2)	0.8 (0.1)	1.4 (0.0)	1.4 (0.1)	0.6 (0.0)	0.6 (0.1)

[a] Microbial C (Cm): total C (Ct).

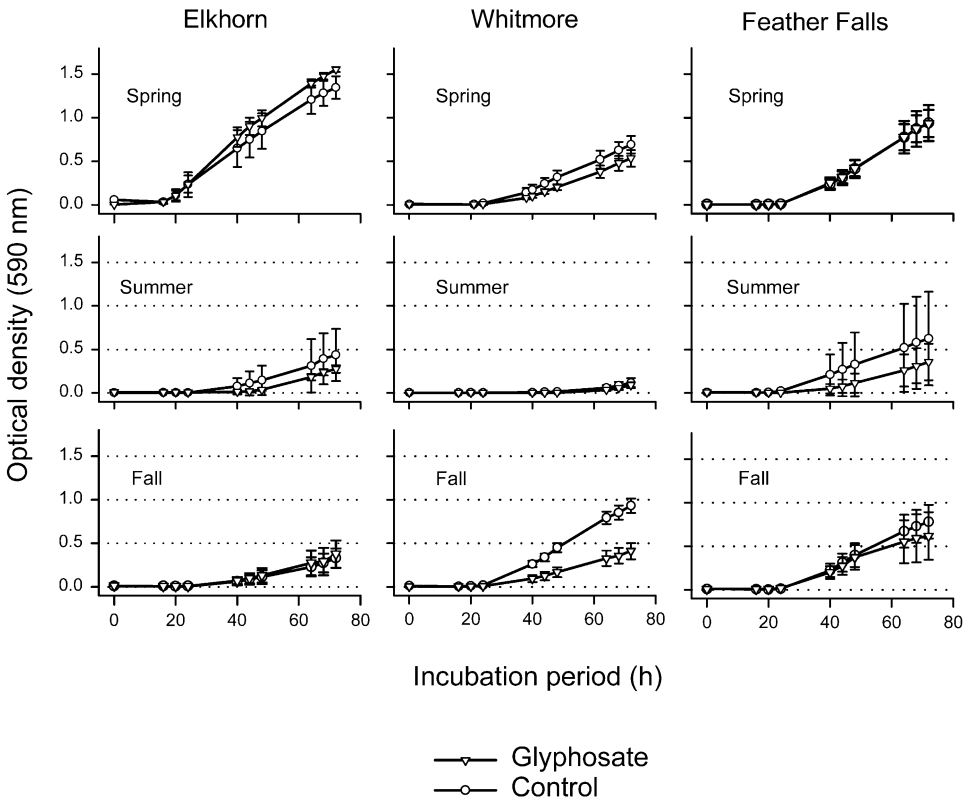

Fig. 3. Average well-color development in Biolog GN plates of soil communities collected seasonally from three ponderosa pine plantations.

Table 4
ANOVA *P* values for microbial characteristics from the field experiment

Source of variation	Microbial biomass	Basal respiration	Metabolic quotient	Cm:Ct[a]	Total bacteria
Site (S)	0.001	0.001	0.004	0.001	0.001
Herbicide (H)	0.476	0.681	0.527	0.005	0.689
S × H	0.162	0.872	0.416	0.018	0.521
Time (T)	0.001	0.001	0.001	0.001	0.016
T × S	0.001	0.001	0.001	0.001	0.001
T × H	0.051	0.107	0.406	0.338	0.738
T × S × H	0.115	0.334	0.333	0.235	0.969

[a] Microbial C (Cm): total C (Ct).

Table 5
Proportional increase in substrate-induced respiration relative to basal respiration for glyphosate and control treatments. Samples were collected in autumn 1998 from the field experiment. Values are means (SD) for SIR/basal respiration

Carbon source (*n*)	Elkhorn		Whitmore		Feather Falls	
	Glyphosate	Control	Glyphosate	Control	Glyphosate	Control
Carbohydrates (9)	1.78 (0.32)	1.51 (0.33)	4.39 (1.23)	3.71 (1.43)	3.64 (1.51)	3.03 (1.15)
Carboxylic acids (8)	1.08 (0.70)	1.23 (0.71)	5.00 (3.64)	4.26 (3.02)	6.66 (6.41)	5.80 (5.62)
Amino acids (9)	1.79 (0.46)	1.78 (0.36)	3.09 (1.35)	3.19 (1.55)	2.98 (0.67)	2.96 (0.90)
Amines (2)	0.75 (0.42)	0.52 (0.25)	1.45 (0.74)	1.84 (0.42)	1.80 (0.30)	1.97 (0.35)
Amides (1)	2.47	2.14	4.02	4.36	3.75	4.85
Polymers (4)	1.54 (0.79)	1.68 (0.70)	2.66 (0.73)	2.63 (0.78)	2.43 (0.65)	2.63 (1.09)
Nucleosides (2)	1.52 (0.23)	1.40 (0.40)	1.44 (0.15)	1.61 (0.07)	1.52 (0.02)	1.69 (0.10)

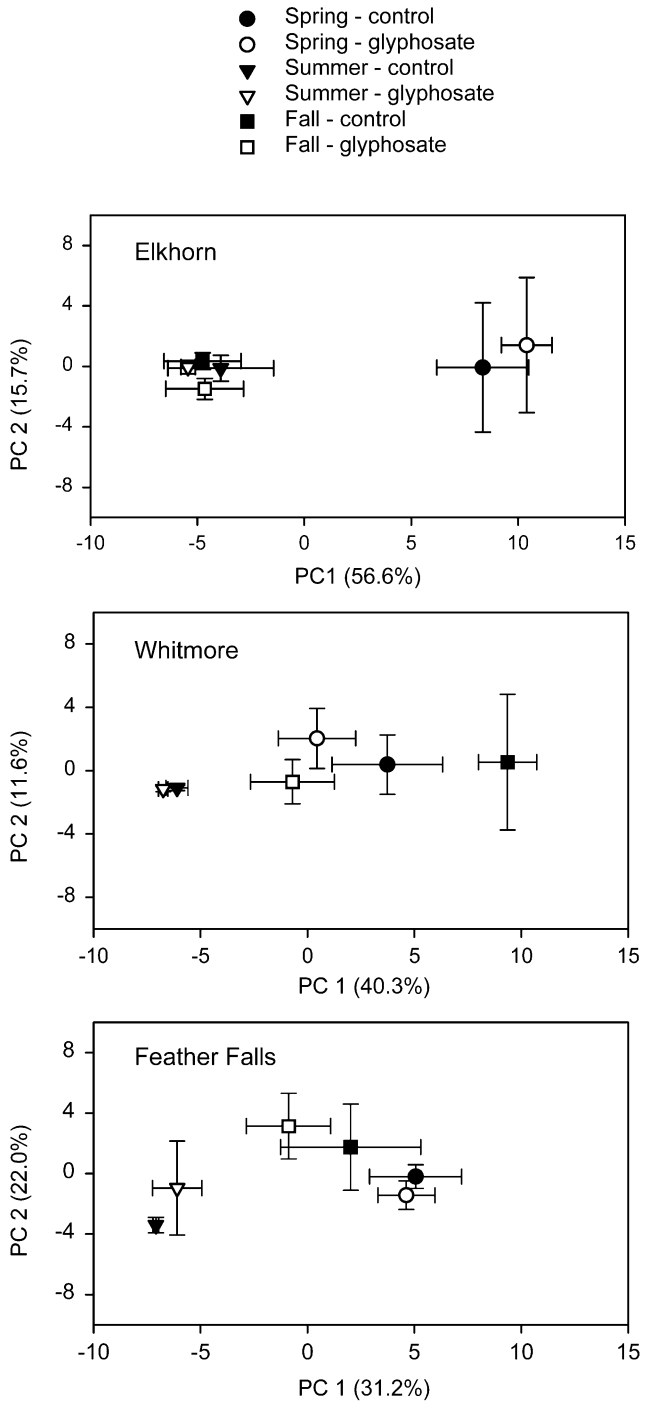

Fig. 4. Principle component analysis of Biolog results from soil communities collected seasonally from three ponderosa pine plantations. Each point represents the mean and standard error ($n = 3$).

from the lowest to highest in northern California. The findings were generally consistent for all indices, soils, and sampling dates: single or repeated applications of glyphosate at the recommended field concentration had little affect on microbial communities.

A major caveat to this finding was the pronounced toxicity of glyphosate in artificial media. Culturable bacteria and fungi were reduced in number or eliminated when extracted from soil and grown on solid media containing glyphosate. Further, the growth rate and metabolic competency of surviving bacteria on Biolog GN plates was reduced by glyphosate. Toxicity in artificial media comes as little surprise, as is predicted by the mode of action of glyphosate. Others studies have found similar reductions in population counts when glyphosate is added to culture media (Christy et al., 1981; Quinn et al., 1988; Santos and Flores, 1995; Krzysko-Lupicka and Orlik, 1997). Typically, supplemental aromatic amino acids are required to support protein synthesis and overcome the inhibition of shikimic acid pathway (Quinn et al., 1988). Of note, the growth decline on Biolog plates was not consistent between soils. The bacterial community from the Elkhorn plantation showed lower tolerance to glyphosate than those from Whitmore and Feather Falls, indicating differences in the diversity of culturable bacteria between soils.

Table 6
Contribution of C, N, and P to the observed stimulation of soil respiration by high concentrations of glyphosate. Each compound was added to soil from the Whitmore plantation at its pre-determined saturation level for microbial respiration. Respiration rates are the mean (SD) of three readings taken during the initial 24 h following substrate addition

Substrate	Respiration (μmol g^{-1} h^{-1})[a]
Glyphosate	0.191 (0.014) a
C	0.165 (0.010) a
C, N	0.166 (0.010) a
C, P	0.183 (0.008) a
C, N, P	0.166 (0.019) a
N	0.042 (0.001) b
P	0.066 (0.003) b
N, P	0.059 (0.006) b
None (control)	0.046 (0.001) b

[a] Column values not sharing a common letter are significantly different at the 0.05 probability level (Tukey test).

Unlike the response in artificial media, no toxicity was expressed when glyphosate was added to soil in laboratory bioassays. Soil respiration at concentrations expected following field application (5–50 mg kg^{-1}) was identical to the respiration rate of the control, regardless of soil type. Plate counts, colony morphology, and metabolic diversity were also comparable between glyphosate and control samples (data not shown). Differences in toxicity between artificial media and soil apparently reflect the chemical nature of glyphosate as both an anti-microbial and a polar compound that is easily inactivated in soil by physiochemical adsorption. This observation supports the conclusion of Quinn et al. (1988) that experiments using artificial, soil-free media are of limited ecological relevance in predicting microbial response to glyphosate in terrestrial systems.

Soil respiration was strongly stimulated by the addition of saturating levels of glyphosate. The increase, which ranged from three- to eight-fold relative to control samples, varied by plantation soil: Whitmore > Feather Falls > Elkhorn.

Interestingly, this pattern follows the same order as their glyphosate-adsorption potential based on oxide and clay content. This is not to suggest a mechanistic link between adsorption and degradation potential in soil, however. Instead, we assume that this relationship is merely coincidental, since the early stages of glyphosate degradation occur almost exclusively in the non-adsorbed, labile phase (Eberbach, 1998). More likely, the observed variation between soils was an expression of differences in the population size of opportunistic organisms capable of degrading glyphosate under saturated conditions. In similar assays, Stratton and Steward (1992) found high concentrations of glyphosate stimulated respiration in an acidic forest soil, and Carlisle and Trevors (1986a) found an increase in aerobic and anaerobic respiration at high concentrations in an agricultural soil. Practical implications from studies of high glyphosate concentrations are unclear. The highest concentration used in our study, for example, was at least 100-fold greater than expected following aerial or directed sprayings, and thus represents only a 'worst-case-scenario' such as a herbicide spill.

Evidence from the nutrient-addition experiment suggests that glyphosate degradation in soil is primarily an energy acquiring process. Glyphosate-induced respiration was equivalent to the respiration rate measured following the addition of an optimum concentration of glucose (Table 3). Nitrogen and P additions, either alone or in combination with glucose, had no additive effect on respiration. Nutrient acquisition cannot be completely ruled out, however, particularly by certain bacterial and fungal species known to utilize glyphosate-containing P (Liu et al., 1991; Dick and Quinn, 1995; Krzysko-Lupicka and Orlik, 1997). A small percentage of organisms may have acquired P or N during glyphosate degradation, yet their activity remained undetected by the community-scale measure of soil respiration.

We found little evidence that repeated field applications of glyphosate were detrimental to microbial populations and processes in mineral soil. Basal respiration, metabolic quotient, total bacteria, metabolic diversity, and mineralizable N were comparable between glyphosate and control treatments at each site and for each sample period. In support of this finding, Busse et al. (2000) found no change in microbial biomass in the litter layer at Whitmore and Feather Falls plantations as a result of vegetation control. The only exceptions we found to this conclusion were reductions in microbial biomass and Cm:Ct at Elkhorn and Whitmore after continuous vegetation control. These declines were relatively small, however, and inconsistent throughout the season.

The similarity in microbial characteristics between field treatments belies the sizeable response in vegetation cover and growth following herbicide application. By the 10th year of the study, understory vegetation cover on control plots was high, ranging from about 25% at Elkhorn to 94% at Whitmore and 110% at Feather Falls (Powers and Reynolds, 1999). Glyphosate-treated plots, in comparison, remained free of understory vegetation for the length of the study. Tree growth was significantly greater on vegetation control plots at each plantation. The Whitmore plantation, for example, had a 300% increase in stand volume by year 10 due to herbicide treatment (Powers and Reynolds, 1999). In addition, the herbicide treatment led to improved water and nutrient availability in the rooting profile and unquantified, yet presumed, changes in root growth and turnover, litter quantity and quality, and microclimate. Nevertheless, synchronicity between plant and microbial responses was absent in the early growth of these plantations. A similar lack of correlation between plant production and microbial characteristics following glyphosate application was reported in a short-term agricultural study (Wardle and Parkinson, 1991).

Two other soil properties, total C and seasonal water content, were unaffected by the repeated herbicide applications. This observation helps shed light on the unresponsive nature of microbial populations to vegetation control since soil C and water are major driving forces of most microbial processes. Evidently, the lack of microbial response was symptomatic of the general tolerance in the surface soil to changes in vegetation. This differs considerably from the findings of Busse et al. (1996). They found significant changes in surface soil properties, including total C, N, and microbial biomass, following 35 years of vegetation control in natural ponderosa pine stands in central Oregon. Differences in site conditions and study length help explain the contrasting results. For example, the soils at our study sites are older, more developed (weathered), and have greater organic matter content and total nutrients, and are thus more apt to tolerate or recover from disturbance. The warmer climate at the California plantations further implies faster mineralization of added C, leading to more rapid equilibrium between C inputs and outputs. Study length may also play a large, untold role. Whether stand development during the next 20–25 years at our plantations will result in changes in microbial indices similar to those seen by Busse et al. (1996) remains to be seen.

Other moderate- to long-term studies involving glyphosate treatment provide interesting comparison to our results. Nine-year, repeated applications of glyphosate on agricultural fields at Rothamsted had no effect on microbial biomass, N mineralization, or nitrification (Hart and Brookes, 1996). Only a short-term decline in CO_2 evolution was found, 56 days after application. In contrast, Munson et al. (1993) found a large increase in nitrification after 4 years of annual glyphosate application in a boreal, mixed-conifer plantation, leading the authors to express concern for potential NO_3 leaching losses. Similar concerns were reported by Vitousek and Matson (1985) in loblolly pine plantations. Herbicide application was credited with increasing net N mineralization, nitrification, and potential nitrate losses due to reduced immobilization of N by microorganisms. In our study, we found no effect of repeated glyphosate

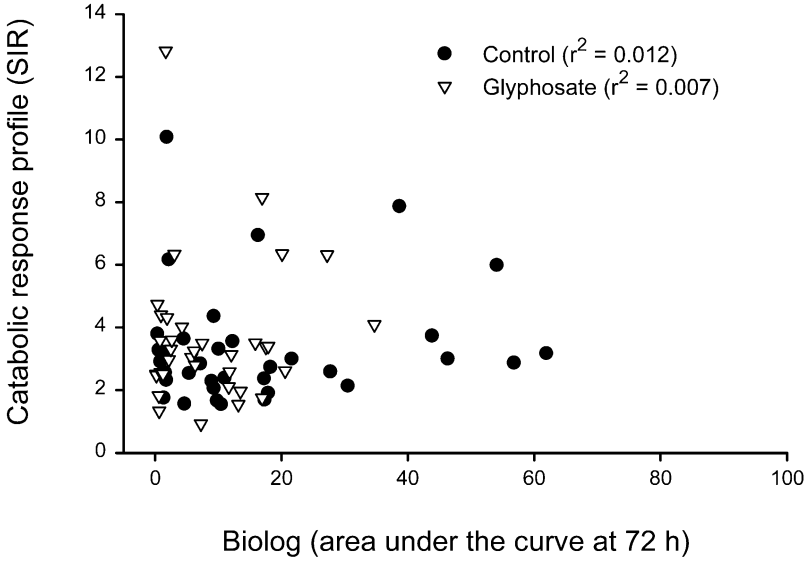

Fig. 5. Relationship between Biolog and catabolic response profile for 36 single-C compounds. Soil communities were sampled from the Whitmore plantation in the autumn of 1998.

applications on mineralizable N, while concerns for nitrate leaching and ground water contamination in these stands are minor given the deep ground-water depths, the lack of nearby streams, and Mediterranean-type climate (Frazier et al., 1990).

Domsch et al. (1983) suggested that non-target effects of pesticides on soil microorganisms are obscured by variability from natural factors. Fluctuations in moisture, temperature, and substrate availability, and the additive effects of time and disturbance (such as land conversion and clearing) overshadow herbicide effects. Our results concur with this concept. Community responses to vegetation control were masked by temporal and spatial effects. For example, basal respiration and metabolic diversity (Biolog) were strongly influenced by time of year and location. Both variables were greatest in the spring when moisture content was near optimal, then declined during summer and recovered partially as soil moisture was replenished by autumn rains. Poor recovery was found at the Elkhorn plantation in the absence of autumn rain. The variables (basal respiration in particular) also varied by site quality, attaining their highest level at Feather Falls, the most productive site, and their lowest level at Elkhorn, the least productive site.

Previous land management was an additional factor that overshadowed the effect of glyphosate on soil organisms. The sites were previously cleared and brush raked prior to planting. The effect on microbial populations was tangible several years after treatment. For example, our study plots had about one-half the microbial biomass and basal respiration compared to adjacent, uncut stands (Busse, unpublished). Evidently, removal or redistribution of organic material and modification of microclimate following clearing and site preparation far surpassed the impact of vegetation control. This observation contrasts with the results of Vitousek and Matson (1985) who found minimal impact of clearcutting on N cycling processes compared to the effect of vegetation control.

A final comment is warranted regarding the two methods used to determine metabolic diversity. Both Biolog and the catabolic response profile method of Degens and Harris (1997) are accepted indices of metabolic diversity by soil bacteria. And although the general conclusions drawn from each method were similar (differences in metabolic diversity between glyphosate and control treatments were minor), the methods were in poor agreement with respect to how the microbial community utilized individual C compounds. Bacterial growth (Biolog) and substrate-induced respiration (catabolic response profile) were compared using 36 compounds in common. No correlation was found between the two methods for herbicide-treated and control soil at the Whitmore plantation (Fig. 5). Similar results were also obtained for the other two sites (Feather Falls: herbicide treatment $r^2 = 0.023$, control treatment $r^2 = 0.001$; Elkhorn: herbicide treatment $r^2 = 0.037$, control treatment $r^2 = 0.019$). Although curious, this finding is not entirely surprising. Biolog is an enrichment technique that compares only culturable bacteria. Catabolic response profile, in contrast, measures the activity of the entire community and avoids the problem of selective enrichment, a common criticism of the Biolog method (Konopka et al., 1998). However, catabolic response profiles are a labor-intensive method, limited in practical use by the number of compounds that can be efficiently tested. While neither method provided a complete picture of metabolic diversity, they were supportive indices of the general conclusion that glyphosate has no consequential affect on soil communities in ponderosa pine plantations.

Acknowledgements

We thank Jim Baldwin for his advice on statistical analyses and for his review of the manuscript. This study was supported by the USDA Forest Service Pesticide Impact Assessment Program (FS-PIAP).

References

Anderson, J.P.E., Domsch, K.H., 1978. A physiological method for the quantitative measurement of microbial biomass in soil. Soil Biology & Biochemistry 10, 215–221.

Bentley, R., 1990. The shikimate pathway—a metabolic tree with many branches. Critical Reviews in Biochemistry & Molecular Biology 25, 307–384.

Bottomley, P.J., 1994. Light microscope methods for studying soil microorganisms. In: Weaver, R.W., Angle, J.S., Bottomley, P.J. (Eds.). Methods of Soil Analysis. Part 2. Microbiological and Biochemical Properties. Soil Science Society of America, Madison, WI, pp. 81–105.

Busse, M.D., Cochran, P.H., Barrett, J.W., 1996. Changes in ponderosa pine site productivity following removal of understory vegetation. Soil Science Society of America Journal 60, 1614–1621.

Busse, M.D., Rappaport, N.G., Powers, R.F., 2000. Tolerance of soil organisms to herbicide applications in ponderosa pine plantations: initial findings. In: Cooper, S.L. (Comp.), Reforestation for the New Millennium: Back to Our Roots. Proceedings of the 21st Annual Forest Vegetation Management Conference, University of California, Shasta County Cooperative Extension, Redding, CA, pp. 33–38.

Calif. Dept. Pesticide Regulation, 2000. Summary of pesticide use report data for 1998. State of Calif., Environmental Protection Agency. p 494.

Carlisle, S.M., Trevors, J.T., 1986a. Effect of the herbicide glyphosate on respiration and hydrogen consumption in soil. Water, Air, and Soil Pollution 27, 391–401.

Carlisle, S.M., Trevors, J.T., 1986b. Effects of the herbicide glyphosate on nitrification, denitrification, and acetylene reduction in soil. Water, Air, and Soil Pollution 29, 189–203.

Carlisle, S.M., Trevors, J.T., 1988. Glyphosate in the environment. Water, Air, and Soil Pollution 39, 409–420.

Chakravarty, P., Chatarpaul, L., 1990. Non-target effect of herbicides: II. The influence of glyphosate on ectomycorrhizal symbiosis of red pine (*Pinus resinosa*) under greenhouse and field conditions. Pesticide Science 28, 243–247.

Christy, S.L., Karlander, E.P., Parochetti, J.V., 1981. Effects of glyphosate on the growth rate of *Chlorella*. Weed Science 29, 5–7.

Degens, B.P., Harris, J.A., 1997. Development of a physiological approach to measuring the catabolic diversity of soil microbial communities. Soil Biology & Biochemistry 29, 1309–1320.

Dick, R.E., Quinn, J.P., 1995. Glyphosate-degrading isolates from environmental samples: occurrence and pathways of degradation. Applied Microbiology & Biotechnology 43, 545–550.

Domsch, K.H., Jagnow, G., Anderson, T-H., 1983. An ecological concept for the assessment of side effects of agrochemicals on soil microorganisms. Residue Reviews 86, 65–105.

Eberbach, P., 1998. Applying non-steady-state compartmental analysis to investigate the simultaneous degradation of soluble and sorbed glyphosate (N-(phosphonomethyl)glycine) in four soils. Pesticide Science 52, 229–240.

Franz, J.E., Mao, M.K., Sikorski, J.A., 1997. Glyphosate: a unique global herbicide. American Chemical Society Monograph 189. American Chemical Society, Washington DC.

Frazier, D.W., McColl, J.G., Powers, R.F., 1990. Soil nitrogen mineralization in a clearcutting chronosequence in a northern California conifer forest. Soil Science Society of America Journal 54, 1145–1152.

Garland, J.L., Mills, A.L., 1991. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. Applied & Environmental Microbiology 57, 2351–2359.

Gianfreda, L., Sannino, F., Violane, A., 1995. Pesticide effects on the activity of free, immobilized and soil invertase. Soil Biology & Biochemistry 27, 1201–1208.

Guckert, J.B., Carr, G.J., Johnson, T.D., Hamm, B.G., Davidson, D.H., Kumagai, Y., 1996. Community analysis by Biolog: curve integration for statistical analysis of activated sludge microbial habitats. Journal of Microbiological Methods 27, 183–197.

Haney, R.L., Senseman, S.A., Hons, F.M., Zuberer, D.A., 2000. Effect of glyphosate on soil microbial activity and biomass. Weed Science 48, 89–93.

Hart, M.R., Brookes, P.C., 1996. Soil microbial biomass and mineralisation of soil organic matter after 19 years of cumulative field applications of pesticides. Soil Biology & Biochemistry 28, 1641–1649.

Insam, H., 1997. A new set of substrates proposed for community characterization in environmental samples. In: Insam, H., Rangger, A. (Eds.). Microbial Communities. Functional versus Structural Approaches. Springer, New York, pp. 259–260.

Konopka, A., Oliver, L., Turco Jr, R.F., 1998. The use of carbon substrate utilization patterns in environmental and ecological microbiology. Microbial Ecology 35, 103–115.

Krzysko-Lupicka, T., Orlik, A., 1997. The use of glyphosate as the sole source of phosphorus or carbon for the selection of soil-borne fungal strains capable to degrade this herbicide. Chemosphere 34, 2601–2605.

Levesque, C.A., Rahe, J.E., 1992. Herbicide interactions with fungal root pathogens, with special reference to glyphosate. Annual Review of Phytopathology 30, 579–602.

Liu, C.-M., McLean, P.A., Sookdeo, C.C., Cannon, F.C., 1991. Degradation of the herbicide glyphosate by members of the family Rhizobiaceae. Applied & Environmental Microbiology 57, 1799–1804.

McBride, M., Kung, K., 1989. Complexation of glyphosate and related ligands with iron (III). Soil Science Society of America Journal 53, 1668–1673.

Morillo, E., Undabeytia, T., Maqueda, C., 1997. Adsorption of glyphosate on the clay mineral montmorillonite: effect of Cu (II) in solution and adsorbed on the mineral. Environmental Science & Technology 31, 3588–3592.

Muller, M.M., Rosenberg, C., Siltanen, H., Wartiovaara, T., 1981. Fate of glyphosate and its influence on nitrogen-cylcing in two Finnish agricultural soils. Bulletin of Environmental Contamination & Toxicology 27, 724–730.

Munson, A.D., Margolis, H.A., Brand, D.G., 1993. Intensive silvicultural treatment: impacts on soil fertility and planted conifer response. Soil Science Society of America Journal 57, 246–255.

Nelson, D.W., Sommers, L.E., 1982. Total carbon, organic carbon, and organic matter. In: Page, A.L., et al. (Eds.), Methods of Soil Analysis. Part 2. 2nd ed. Agonomy Monograph 9. ASA and SSSA, Madison, WI, p. 539–579.

Olson, B.M., Lindwall, C.W., 1991. Soil microbial activity under chemical fallow conditions: effects of 2,4-D and glyphosate. Soil Biology & Biochemistry 23, 1071–1075.

Piccolo, A., Celano, G., Arienzo, M., Mirabella, A., 1994. Adsorption and desorption of glyphosate in some European soils. Journal of Environmental Science & Health B29, 1105–1115.

Powers, R.F., 1980. Mineralizable soil nitrogen as an index of nitrogen availability to forest trees. Soil Science Society of America Journal 44, 1314–1320.

Powers, R.F., Ferrell, G.T., 1996. Moisture, nutrient, and insect contraints on plantation growth: the 'Garden of Eden' study. New Zealand Journal of Forest Science 83, 11–24.

Powers, R.F., Oliver, W.W., 1978. Site classification of ponderosa pine under stocking control in California. USDA Forest Service Research Paper, PSW-128.

Powers, R.F., Reynolds, P.E., 1999. Ten-year responses of ponderosa pine plantations to repeated vegetation and nutrient control along an

environmental gradient. Canadian Journal of Forest Research 29, 1027–1038.

Powers, R.F., Reynolds, P.E., 2000. Intensive management of ponderosa pine plantation: sustainable productivity for the 21st century. Journal of Sustainable Forestry 10, 249–255.

Quinn, J.P., Peden, J.M.M., Dick, R.E., 1988. Glyphosate tolerance and utilization by the microflora of soils treated with the herbicide. Applied Microbiology & Biotechnology 29, 511–516.

Roslycky, E.B., 1982. Glyphosate and the response of the soil microbiota. Soil Biology & Biochemistry 14, 87–92.

Rueppel, M.L., Brightwell, B.B., Schaefer, J., Marvel, J.T., 1977. Metabolism and degradation of glyphosate in soil and water. Journal of Agriculture and Food Chemistry 25, 517–528.

Santos, A., Flores, M., 1995. Effects of glyphosate on nitrogen fixation of free-living heterotrophic bacteria. Letters in Applied Microbiology 20, 349–352.

SAS, 1995. SAS User's Guide, Version 6.0. SAS Institute, Cary, NC.

Stratton, G.W., Steward, K.E., 1992. Glyphosate effects on microbial biomass in a coniferous forest soil. Environmental Toxicology & Water Quality 7, 223–236.

Vitousek, P.M., Matson, P.A., 1985. Disturbance, nitrogen availability, and nitrogen losses in an intensively managed loblolly pine plantation. Ecology 66, 1360–1376.

Vitousek, P.M., Andariese, S.W., Matson, P.A., Morris, L., Sanford, R.L., 1992. Effects of harvest intensity, site preparation, and herbicide use on soil nitrogen transformations in a young loblolly pine plantation. Forest Ecology & Management 49, 277–292.

Wardle, D.A., 1995. Impacts of disturbance on detritus food webs in agroecosystems of contrasting tillage and weed management practices. Advances in Ecological Research 26, 105–185.

Wardle, D.A., Parkinson, D., 1991. Relative importance of the effect of 2,4-D, glyphosate, and environmental variables on the soil microbial biomass. Plant & Soil 134, 209–219.

Wardle, D.A., Parkinson, D., 1992. Influence of the herbicides 2,4-D and glyphosate on soil microbial biomass and activity: a field experiment. Soil Biology & Biochemistry 24, 185–186.

Zibilske, L.M., 1994. Carbon mineralization. In: Weaver, R.W., Angle, J.S., Bottomley, P.J. (Eds.). Methods of Soil Analysis. Part 2. Microbiological and Biochemical Properties. Soil Science Society of America, Madison, WI, pp. 835–906.