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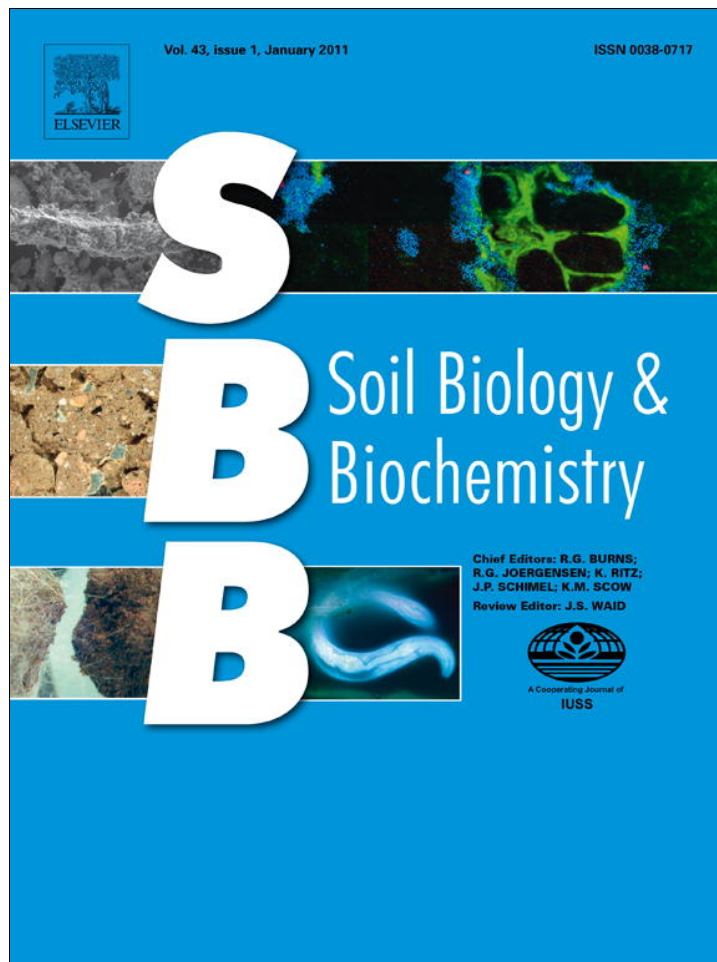

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Soil microbial communities resistant to changes in plant functional group composition

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A B S T R A C T

The soil community is an often ignored part of research which links plant biodiversity and ecosystem functioning despite their influence on numerous functions such as decomposition and nutrient cycling. Few consistent patterns have been detected that link plant and soil community composition. We used a removal experiment in a northern Canadian grassland to examine the effects of plant functional group identity on soil microbial community structure and function. Plant functional groups (graminoids, legumes and forbs) were removed independently from plots for five growing seasons (2003–2007) and in the fifth year effects on the soil microbial community were examined using substrate-induced respiration (SIR – a measure of metabolic diversity) and phospholipid fatty acid analysis (PLFA – a measure of microbial community composition). Removal treatments were also crossed with both a fertilizer treatment and a fungicide treatment to determine if effects of functional group identity on the soil community were context dependent. Plant functional group identity had almost no effect on the soil microbial community as measured by either SIR or PLFA. Likewise, soil properties including total carbon, pH, moisture and nutrients showed a limited response to plant removals in the fifth year after removals. We found a direct effect of fertilizer on the soil community, with fertilized plots having decreased metabolic diversity, with a decreased ability to metabolize amino acids and a phenolic acid, but there was no direct soil microbial response to fungicide. We show that in this northern Canadian grassland the soil microbial community is relatively insensitive to changes in plant functional group composition, and suggest that in northern ecosystems, where plant material is only slowly incorporated into the soil, five growing seasons may be insufficient to detect the impact of a changing plant community on the soil microbes.

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1. Introduction

Current rates of species extinctions are causing concern in both the scientific and the public community. Human alterations to ecosystems and increases in invasive species have eliminated many native species or drastically changed their abundance (Chapin et al., 2000). Much research has examined the consequences of decreased diversity on ecosystems, forming the body of research termed biodiversity ecosystem functioning (BDEF), but there is an on-going debate over the interpretation of the results and conclusions (see reviews by Balvanera et al., 2006; Duffy, 2009; Hooper et al., 2005). Studies investigating how future species loss will affect complex ecosystems are likely to provide valuable information regarding future management.

The soil microbial community is an often ignored component of research examining the effects of plant biodiversity loss on ecosystem functioning (Balvanera et al., 2006). Soil organisms have a large impact on decomposition (Wardle et al., 1999), nutrient cycling (Cavigelli and Robertson, 2000), and influence many aboveground processes, such as plant community composition (Bezemer et al., 2006; van der Heijden et al., 2008; Wardle, 2005). The belowground community is highly species diverse and adds a complex and poorly understood aspect to ecosystem studies, and the small portion of BDEF research that has considered the belowground community has shown varied results. Results depend on which microbial process is measured and the methods chosen to analyze the soil microbial community (Niklaus et al., 2006; Wardle et al., 1999; Zak et al., 2003), the experimental design (Balvanera et al., 2006), site location (Bezemer et al., 2006) and plant species identity (Chen and Stark, 2000).

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There has been little success in detecting consistent patterns that describe plant diversity effects on the soil microbial community. Studies in experimental grasslands have reported increased diversity of soil bacteria with increasing plant diversity (Stephan et al., 2000) and increased microbial biomass with higher plant diversity (Spehn et al., 2000; Zak et al., 2003). Wardle et al. (2003) and Niklaus et al. (2006), however, detected no change in microbial biomass with plant species or functional group diversity in microcosm communities. Finally, in a removal experiment on re-establishing vegetation in experimental bare patches in New Zealand pastures there was an idiosyncratic relationship between the soil microbial community structure and function, and plant functional group diversity and composition (Wardle et al., 1999).

Plants interact with the soil microbial community in many ways. Nutrients required by soil microbes often come from plant litter or through root exudation (Grayston et al., 1998). Certain soil microorganisms form host-specific relationships with plant groups or species (Bever, 2003), such as legumes and nitrogen-fixing bacteria. Roots also physically alter the soil structure creating varying microhabitats suitable for different microbes (Bardgett et al., 2005a,b). Mycorrhizal associations with plant roots add another dimension to the plant–soil–microbe system because mycorrhizae can directly and indirectly influence soil microbes (see below). Plant traits may also influence soil community composition by impacts on soil carbon sequestration, via differences in exudates, and litter quality and quantity (De Deyn et al., 2008), or through changes in short-term C-cycling (Ward et al., 2009). The characteristic plant traits that define different plant functional groups (such as differences between legumes and graminoids in their C:N ratio) are likely to influence their differing effects on a variety of soil properties that could feedback to soil microbial community composition.

The effects of plant community composition on the structure of the microbial community may also depend on environmental conditions. Predicted future environmental changes include not only increased temperatures but also increased nutrient levels due to nitrogen deposition and increased decomposition (Shaver et al., 2000). These changes will be especially important in northern ecosystems where decomposition rates and nutrient cycling are typically slower due to lower temperatures (Shaver et al., 2000). There are many ways this increase in nutrients could affect the soil community. Although the soil community is typically thought of as carbon limited, studies have also reported their potential nitrogen limitation (Chen and Stark, 2000; Wagener and Schimel, 1998). Furthermore, nitrogen requirements appear to vary among soil microorganisms (Schimel et al., 2005). For example, soil communities under conditions of high nutrients tend to be bacterial-dominated whereas lower-nutrient soils tend to support fungal-dominated communities (Wardle, 2005).

The nature of the microbial community may also be influenced by mycorrhizal fungi. The fungi involved in these associations form an intimate symbiosis with plant roots and therefore have immediate access to plant root exudates. Mycorrhizae can alter the chemical composition and relative abundance of these exudates, which are a source of nutrients for many soil organisms (Artursson et al., 2006). Andrade et al. (1997) reported higher numbers of bacteria in areas of the roots not occupied by arbuscular mycorrhizal (AM) fungi, implying a greater release or higher quality of root exudates from those areas. The physiological state of the mycorrhizal hyphae has also been shown to allow associations with different bacterial strains (Toljander et al., 2005). As both mycorrhizal status and soil nutrient levels may affect the plant–soil relationships which influence soil community composition, the effects of plant functional group identity on soil communities may be context dependent.

The objective of this study was to investigate how the plant functional group identity influences the belowground microbial community. To examine this question, we conducted a removal experiment, where each of three functional groups (graminoids, forbs and legumes) was independently removed from a northern Canadian grassland. These removals were conducted within two different environmental contexts, a fertilizer treatment and a fungicide treatment (to reduce mycorrhizal colonization), to determine if the influence that the plant composition had on the soil microbial community was context dependent.

The effects of plant functional group identity on the soil community were examined in the fifth year of this long-term functional group removal experiment. Results from the first four years indicate that plant functional group identity affects soil and ecosystem properties, including soil moisture, soil nutrient availability and light interception (McLaren and Turkington, 2010). In this study grasses had a larger influence on ecosystem properties than expected based on their biomass contribution to the community; removals of grasses increased both soil moisture and soil nitrogen, but decreased soil phosphorus (McLaren and Turkington, 2010) which may, in turn, influence the structure of the soil microbial community. Thus, in the fifth year only we assessed changes in the microbial community. This was done in two ways: the metabolic function was analyzed using substrate-induced respiration (SIR) and soil microbial community structure was analyzed using phospholipid fatty acids (PLFA).

2. Methods and materials

This removal experiment was part of a larger experiment examining the role of plant functional group identity in determining various ecosystem functions. Detailed methods are described in McLaren and Turkington (2010), and are summarized below.

2.1. Site description

The study area is a relatively dry grassland near Kluane Lake in the south-western Yukon in northern Canada (61°04.218 N 138°23.018 W). The area receives a mean annual precipitation of ca. 230 mm, about half of which falls as rain during the summer, but also includes an average annual snowfall of about 100 cm. The grassland is surrounded by a closed to relatively open spruce forest community dominated by *Picea glauca* (Moench) Voss. Grassland species were divided into three functional groups, namely, graminoids (grasses and sedges), forbs, and legumes. The grassland is dominated by *Poa glauca* Vahl and *Carex stenophylla* Wahlenb. ssp. *eleocharis* (Bailey) Hultén, and also contains many non-leguminous forbs (dominated by *Erigeron caespitosus* Nutt., *Artemisia frigida* Willd.), and legumes (dominated by *Oxytropis campestris* (L.) DC.); all nomenclature follows Cody (1996).

2.2. Experimental plant communities

Experimental plots were established in May 2003 and maintained annually for 5 years through the 2007 growing season. The experiment was a $4 \times 2 \times 2$ fully crossed factorial design (4 removal treatments, +/0 fertilizer, +/0 fungicide). Each of the 16 treatments was replicated 5 times, resulting in a total of 80 plots. The 1×1 m plots were contained within an area of approximately 0.5 Ha, and were arranged in a constrained random basis to ensure all plots contained representatives from each functional group.

There were four removal treatments: independent removal of each of the three functional groups (graminoids, forbs and legumes) and a no-removal control. In 2003, plants were removed from the plots using Round-up™ glyphosate, a non-selective

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herbicide. Herbicide was painted precisely to the leaves (minimizing contact of Round-up with the soil) and once plants had visibly yellowed, stems of selected plants were clipped at soil level and removed from the plots. Initial biomass removals differed between removal treatments, with the most biomass being removed with forb removals (40.76 g m^{-2} ± 3.83 SE), followed by graminoids (30.55 g m^{-2} ± 2.08 SE), and the legumes (10.08 g m^{-2} ± 1.23 SE) (McLaren and Turkington, 2010). Removal treatments were maintained in subsequent growing seasons, but species from other functional groups were allowed to invade the newly available space created by the removals.

Fertilizer and fungicide treatments were applied upon completion of the removals (July 20) in 2003 and in early-June of each subsequent year. Fertilizer was applied each year to half the plots in granular form at a rate of 17.5 g N m^{-2}, 5.8 g P m^{-2} and 5.8 g K m^{-2}. This application rate was used to be consistent with many other studies being done in the area (e.g. Turkington et al., 1997; Turkington et al., 2002). The fungicide Benlate™ (active ingredient benomyl) was applied to half of the plots as a soil drench (2 Lm^{-2}) every two weeks each year from early-June to mid-August at a rate of 2.5 g benomyl m^{-2} per application. Plots that did not receive fungicide received an equivalent amount of water. Benomyl applications reduced mycorrhizal colonization rates in bulked root samples from each plot from 50% to less than 10% of root intersections (J. McLaren, unpublished data); colonization rates for individual species were not determined.

2.3. Response measurements

Soil for microbial community analysis was collected on 4 July 2007, in the fifth year after the removals were established. Three 2 cm diameter cores were taken to a depth of 8 cm from each plot (a few samples were taken to only 5 cm when the random sampling location was placed over shallow soil), combined, homogenized and sieved through a 2 mm sieve. Soil microbial community structure and function was analyzed using two techniques. The first, SIR, analyzed the functional potential or catabolic diversity of the community. This method analyzes the fast-growing, culturable fraction of microbial community (Leckie, 2005). Soil samples were analyzed using MicroResp™ plates, following the protocol described by Campbell et al. (2003). The MicroResp system creates a metabolic profile of the microbial community based on the ability of the community to metabolize a series of 11 different carbon sources (Monosaccharides: glucose, fructose; disaccharides: sucrose, maltose; amino acids: lysine, ornithine, phenylalanine; organic acids: tartaric acid, citric acid; phenolic acids: caffeic acid, ferulic acid) determined by the amount of CO_2 produced after a 6 h incubation period at 20 °C. There were 8 replicates of each carbon source per experimental plot. These carbon sources were chosen to represent compounds likely to be found in the soil and used by soil microorganisms, such as compounds from plant root exudates (Grayston et al., 2004).

The second technique, PLFA, analyzes the entire microbial community alive in the soil at the time of sampling (Leckie, 2005) using the abundance of different membrane lipids, the composition of which varies in different organisms (Table 1). The procedure used was modified from Bligh and Dyer (1959) and Frostegård et al. (1993). Membrane lipids were extracted from about 2 g of freeze-dried soil, and converted to fatty acid methyl esters (FAMEs) by methylation (Kirk et al., 2004; Leckie, 2005). A community profile was obtained by passing the final samples through a gas chromatograph (Agilent 6890N GC with an Agilent 5973N mass selective detector), giving the amount of each fatty acid analyzed (nmol PLFA g^{-1} of soil). The fungal to bacterial ratio was calculated using i15:0, a15:0, i16:0, a17:0, cy17:0, 18:1ω7c and cy19:0 to represent bacteria and 18:2ω6,9 to represent fungi (Bååth and Anderson, 2003; Bardgett et al., 1996). PLFA nomenclature follows Steer and Harris (2000).

We also collected aboveground plant biomass, soil moisture, and nutrient supply rates in 2007, in the same manner as 2003–2006 measurements, as described in McLaren and Turkington (2010). In brief, aboveground biomass was determined using a non-destructive point-intercept method. Total leaf hits of all species were determined in mid-July at 100 points arranged in a 10 × 10 grid in a 1 m^2 quadrat. We used a series of regression equations that equated the biomass of each species with the total number of leaf hits (McLaren and Turkington, 2010) to determine biomass of each species for each plot and these values were summed to determine total aboveground biomass.

Soil moisture (%) was measured using a water content sensor (Hydrosense Water content measurement system, Campbell Scientific, Australia) at a depth of 10 cm at peak-flowering (mid-July) 2007. Two measurements were taken in each plot, and the average of these measurements was used in analysis. Soil organic content was based on loss of weight on ignition at 500 °C.

Nutrient supply rates were estimated using ion exchange membranes (Plant Root Simulator (PRS)™-probes; Western Ag Innovations Inc., Saskatoon, Canada) using separate cation and anion-exchange resin membranes. Two probes of each type were used in each plot and pooled for analysis to account for soil heterogeneity. The PRS™-probes were inserted into the soil in late May and remained until the end of the growing season (mid-August) to measure in situ nutrient supply rates. Probes were analyzed by Western Ag Innovations Inc., Saskatoon, Canada, for NO_3^-, NH_4^+, P, K, S, Ca, Mg, Mn, Fe, Cu, Zn, B, Al, and Pb.

2.4. Analyses

Total plant biomass was analyzed as a 3-factor ANOVA, with 3 main plot factors, functional group removals, fertilizer and

Table 1
Phospholipid fatty acids (PLFA) used for analysis of microbial communities and the soil biota group they represent. Common refers to a general PLFA that is present in most microbes.

Gram (+) bacteria	Gram(−) bacteria	Total bacteria	Actino-bacteria	Arbuscular Mycorrhiza	Saprophytic fungi	Fungi	Common
i15:0	i16:1ω7c	15:0	16:0ω6m/10Me17:0	16:1ω5c	18:2ω6,9	18:1ω9c	16:0
a15:0	i17:1ω8c	17:0	17:0ω7m/10Me18:0				
i16:0	cy17:0		18:0ω8m/0Me19:0				
16:1ω9c	18:1ω7c						
16:1ω7	18:1ω5c						
i17:0	cy19:0						
a17:0							
18:0							
18:1ω9c							

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Table 2
Summary of ANOVA for total plant biomass (g m^{-2}) per plot and MANOVA for soil variables (soil carbon, soil moisture, pH and nutrients). Values in bold are significant ($p < 0.05$, Wilks' Lambda). Degrees of freedom are reported as (df treatment, df error).

	Total biomass			Soil variables		
	df[a]	*F*	*p*	df[a]	*F*	*p*
Functional group removal	3, 76	**11.17**	**<0.001**	42,152	**1.91**	**0.003**
Fertilizer	1, 78	**12.29**	**0.001**	14,51	**69.06**	**<0.001**
Fungicide	1, 78	2.06	0.075	14,51	**2.11**	**0.027**
Removal × Fertilizer	3, 76	3.38	0.115	42,152	1.11	0.316
Removal × Fungicide	3, 76	0.39	0.761	42,152	0.87	0.687
Fertilizer × Fungicide	1, 78	0.08	0.185	14,51	**2.35**	**0.013**
Removal × Fertilizer × Fungicide	3, 76	0.08	0.968	42,152	1.12	0.312

[a] Degrees of freedom are reported as (df treatment, df error).

fungicide. Functional group biomass was analyzed using a 3-way MANOVA on all functional groups followed by a 3-way ANOVA on each functional group with *p*-values adjusted using a Bonferonni correction. Soil response variables were analyzed using a 3-way MANOVA on all variables, and if main plot factors were significant this was followed by a 3-way ANOVA on each soil variable with *p*-values adjusted using a Bonferonni correction.

Both SIR for all independent C sources, and PLFA for all fatty acids were analyzed with a 3-way MANOVA, and if main plot factors were significant this was followed by a 3-way ANOVA on each C source or fatty acid respectively with *p*-values adjusted using a Bonferonni correction.

For all variables, if a significant effect of removal treatment was detected, removals were compared using a Tukey's comparison of all means. Data were transformed as required to satisfy the assumptions of normality and multivariate normality. All statistical analyses were performed using SAS 9.1 for Windows.

3. Results

3.1. Aboveground variables

Plant functional group removal and fertilization, but not fungicide, significantly affected total plant biomass (Table 2). Forb and legume, but not grass, removal resulted in a lower total biomass compared with the controls (Fig. 1). Fertilization increased total aboveground biomass (mean g m^{-2} ± SE: Fertilized 94.41 ± 3.53; Unfertilized 81.71 ± 2.47). When total biomass was separated into the respective functional groups, fertilization significantly affected functional group biomass (MANOVA $F_{3,74} = 2.82$, $p = 0.045$); legume biomass was significantly less when fertilized (ANOVA $F_{1,78} = 35.35$, $p < 0.001$), although for the other two functional groups fertilization had no effect. Fungicide had no effect on the biomass of any functional group (MANOVA $F_{3,74} = 0.03$, $p = 0.99$).

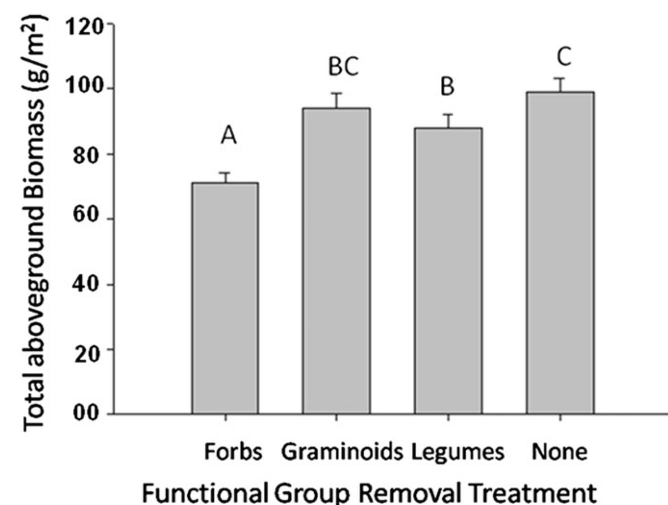

Fig. 1. Mean total aboveground biomass in plots (+1SE) after 5 years of functional group removals averaged across fungicide and fertilizer treatments ($n = 80$). Bars with different letters are significantly different (Tukey's comparison of all means).

3.2. Soil variables

Soil variables (soil moisture, pH, nutrients and soil organic matter) were significantly affected by functional group removal (Table 2), but after Bonferonni corrections no independent soil variable was significantly affected by removal treatments (Table 3). Fertilization also affected soil variables (Table 2); fertilization decreased soil moisture and soil pH, and affected many soil nutrients; total N, NH^{4+}, K, P, Fe, and Mn increased, whereas Ca and Mg decreased (Table 3). There was a significant fertilization × fungicide interaction for total N (Table 3) where fungicide only increased Total N when plots were not fertilized (Fig. 2).

3.3. Microbial community structure

Functional group removal had no significant effects on the microbial community's metabolic diversity (i.e., the number of C sources utilized by the microbial community) examined through SIR profiles (Table 4). Fertilization, but not fungicide, affected SIR profiles (Table 4). Fertilization decreased the ability of the soil community to metabolize amino acids (lysine, ornithine, and phenylalanine) and a phenolic acid (caffeic acid) (Table 5, Fig. 3).

Neither functional group removal nor fungicide treatments significantly changed PLFA profiles, but fertilization had a significant effect on PLFA profiles (Table 4) because one fatty acid, 18:1ω7cm/10Me19:1ω7c, which is used as a representation of total microbial biomass, increased with fertilizer ($F_{3,76} = 4.41$, $p = 0.0397$), although this increase was not significant after Bonferonni corrections. The ANOVA on fungal:bacterial ratios also gives no significant effect of any treatment ($F_{3,76} = 1.62$; $p = 0.1076$).

4. Discussion

The primary objective of this research was to determine the influence of plant functional groups on the structure and function of the soil microbial community and to examine the context-dependency of these effects. We have shown that the soil microbial community was quite insensitive to removal of plant functional group after 5 years. Neither overall metabolic profiles as examined by SIR nor microbial community structure as examined by PLFA showed any significant changes with any functional group removal treatment.

This lack of response to removals by the soil microbial community is likely not solely due to re-growth by the remaining functional groups. Five years after the initial removals, plots where grasses had been removed had fully compensated for the loss of biomass due to removals. However, in plots that had forbs or legumes removed there was no full compensation of biomass, yet there was also no effect on soil microbial communities. It was surprising that graminoid removal plots recovered more rapidly than plots from which legumes were removed, as the initial removal of biomass was much smaller in the legume removal treatments (McLaren and Turkington, 2010). Legumes may have a disproportionately large impact on ecosystem properties such as vegetation cover, plant composition and nitrogen retention (Spehn

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Table 3
Summary of ANOVA for the effects of functional group removals on soil properties. Values in bold are significant ($p < 0.05/14$, Wilks' Lambda with Bonferonni Correction).

	Functional Group Removal (df = 3, 76)		Fertilizer (df = 1, 78)		Fungicide (df = 1, 78)		Fertilizer × Fungicide (df = 3, 76)	
	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Organic content	0.84	0.475	0.06	0.812	0.20	0.659	0.03	0.859
Soil moisture	0.56	0.641	**33.68**	**<0.001**	7.21	0.009	5.02	0.029
pH	2.09	0.111	**228.12**	**<0.001**	0.00	0.986	0.55	0.462
Total N	0.33	0.800	**274.94**	**<0.001**	**20.65**	**<0.0001**	**18.55**	**<0.0001**
NH_4^+	3.09	0.033	**12.00**	**0.001**	3.98	0.050	0.66	0.418
Ca	0.22	0.885	**13.33**	**0.001**	0.82	0.369	0.14	0.714
Mg	0.55	0.648	**17.36**	**<0.001**	0.77	0.384	0.12	0.735
K	3.53	0.020	**32.12**	**<0.001**	0.21	0.649	0.10	0.758
P	0.44	0.725	**380.50**	**<0.001**	1.10	0.298	2.17	0.146
Fe	2.05	0.110	**9.44**	**0.003**	0.00	0.948	0.59	0.444
Mn	1.1	0.354	**55.94**	**<0.001**	0.82	0.367	1.71	0.196
Zn	2.59	0.060	6.94	0.011	0.91	0.345	0.27	0.605
Bo	1.3	0.281	8.39	0.005	0.08	0.780	0.00	0.956
Al	3.48	0.021	0.91	0.345	0.73	0.396	0.45	0.396

et al., 2005; Stephan et al., 2000), likely due to the benefit gained by other plants from the nitrogen fixation by legumes.

During the first four years of this experiment plant functional group identity, especially graminoids, had significant effects on numerous soil variables including soil moisture, light interception, and numerous soil nutrients (pH and soil carbon were not measured in earlier years) (McLaren and Turkington, 2010), but none of these responses were detected in year 5. The earlier effects of removals may have been transient responses that disappear over the longer term as the remaining functional groups compensate for the removals. By year 5, the plant functional groups remaining may have compensated for the role of the functional group removed, implying little importance of plant functional group identity in determining soil properties over the long term. Alternatively, the presence of effects in years 1–4 and the lack of effects in year 5 may have been simply due to the lower statistical power associated with analyzing functional group effects in a single year. We collected data on soil variables in year 5 independently, however, in an attempt to correlate changes in microbial community composition with functional group-driven changes in ecosystem properties.

The manipulations imposed on this system were quite severe: for five years an entire plant functional group was absent from designated plots and we were surprised that so few treatment effects were detected on the soil community. There are many factors that can potentially influence the soil microbial community: plant community composition (Stephan et al., 2000), plant species identity (Grayston et al., 1998), soil chemical and physical properties (Hamman et al., 2007), land use history (Buckley and Schmidt, 2001), and the soil food web community (Wardle, 2005). In this stress-tolerant habitat no changes in the soil microbial community from plant manipulations were detected by the methods used, or it may take a longer period of time to produce detectable changes. Other studies have also shown a lack of response in the soil microbial community, as indicated by SIR and RNA analysis, to changes in the plant community (Buckley and Schmidt, 2001; Wardle et al., 1999; Wardle et al., 2003). Wardle et al. (2003) artificially assembled plant communities consisting of the same functional groups used in our study and failed to detect any effect of plant functional group richness (1 or all 3) or of plant species richness within a functional group (1 or 3 species) on SIR or PLFA profiles, consistent with our results. Wardle et al. (1999) also did a 3-year functional group removal experiment, and successfully detected significant effects on the soil community only when all plants were removed. Alternatively, effects of plant functional group removals on the soil microbial community may have been transient and disappeared early after removal treatments were applied. Urcelay et al. (2009) also found few effects of plant functional group removals on the mycorrhizal community except for transient effects shortly after the removals. This indicates the AMF community in their study is resilient to change in plant functional groups, or was only responding to the short-term physical disturbance of removals. In our study, a potential flush of nutrients due to root decay following removal treatments may have initially affect both soil conditions and the microbial community, which

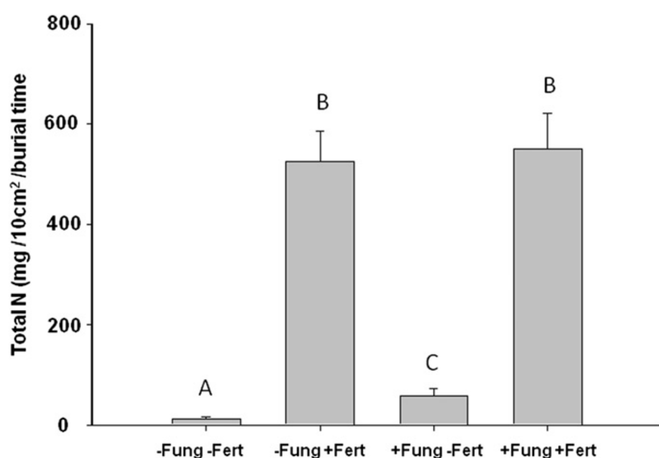

Fig. 2. The effects of 5 years of fungicide (Fung) and fertilizer (Fert) on mean (+1SE) total soil N (across removal treatments, $n = 80$). Bars with different letters are significantly different (Tukey's comparison of all means).

Table 4
Summary of MANOVA for SIR profiles and PLFA profiles of the soil microbial community in response to functional group removal, fertilization and fungicide application. Values in bold are significant ($p < 0.05$, Wilks' Lambda).

	SIR			PLFA		
	df[a]	*F*	*p*	df[a]	*F*	*p*
Functional group removal	33, 145	1.03	0.435	69,126	1.31	0.093
Fertilizer	11, 49	**2.99**	**0.004**	23,42	**4.31**	**<0.001**
Fungicide	11, 49	1.02	0.445	23,42	1.69	0.069
Removal × Fertilizer	33, 145	0.73	0.849	69,126	1.23	0.158
Removal × Fungicide	33, 145	0.99	0.485	69,126	1.04	0.527
Fertilizer × Fungicide	11, 49	0.32	0.978	23,42	0.79	0.725
Removal × Fertilizer × Fungicide	33, 145	1.32	0.136	69,126	0.85	0.774

[a] Degrees of freedom are reported as (df treatment, df error).

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Table 5
Summary of ANOVA for fertilization effect on independent carbon sources in the SIR profiles. Values in bold are significant ($p < 0.05/11$, Wilks' Lambda with Bonferonni Correction). There is 1,78 df in all cases.

Treatment	Type of C source	*F*-value	*p*
Glucose	Monosaccharide	0.07	0.786
Fructose	Monosaccharide	0.90	0.346
Sucrose	Disaccharide	3.10	0.083
Maltose	Disaccharide	3.25	0.076
Lysine	Amino acid	**9.25**	**0.004**
L-Ornithine	Amino acid	**14.96**	**0.000**
L-Phenylalanine	Amino acid	**8.76**	**0.004**
Tartaric acid	Organic acid	5.57	0.021
Citric acid	Organic acid	4.99	0.029
Caffeic acid	Phenolic acid	**12.30**	**0.001**
Ferulic acid	Phenolic acid	2.73	0.104

subsequently recovered from this disturbance. However, there was no difference between the first four years in the effect of removals on soil nutrients (McLaren and Turkington, 2010), suggesting that root decomposition following removal treatments is likely not to strongly influence these results.

Two of the main methods by which plants can influence the soil microbial community are through litter inputs and root exudation. Root exudates are more readily decomposed by the microbial community than plant litter. If the soil properties in this system are influenced mostly by the quality or identity of the litter rather than by the nature of root exudates, it might be expected that a change in the soil properties caused by plant composition would take longer to manifest (Wardle et al., 1999). Further, plant root litter, which may be incorporated into the soil more quickly than leaf litter, is often more similar in N concentration between plant functional groups than the corresponding leaf litter (Craine et al., 2005). Lavorel et al. (2007) and De Deyn et al. (2008) suggested that in low-nutrient habitats with short growing seasons, plants will produce fewer root exudates and also have lower quality litter that takes longer to decompose (Chapin, 2003). In a greenhouse experiment examining the importance of plant presence or litter presence on soil microbial activity (SIR), the effect of litter was found to be much greater (Dornbush, 2007).

Soil organic matter, or detritus, is a more stable, persistent, source of energy than primary production (Moore et al., 2004) and may stabilize soil communities against changes in plant community structure. For example, the buffering ability of high soil carbon was suggested as the reason behind a lack of effects of plant community composition on the soil food web by Wardle et al. (1999) (13% soil carbon) and Dornbush et al. (2008) (3% soil carbon), and our soil carbon falls within the range presented by these two papers (7%, unpublished data). Soil organic matter was unaffected by the treatments in our study and it is quite likely that 5 years of treatment may be insufficient to produce changes to carbon feedbacks to the soil community.

We only sampled the soil microbial community at a single time point during the 5 years of this removal experiment. Both the nature and importance of plant–soil organism interactions can be very dependent on temporal scales, especially considering the differences between plants and microbes in their body size, life history, and habitat size (Bardgett et al., 2005a,b). Soil microbial community structure can be closely linked to variation in carbon (Wardle et al., 1999). The influence of plants on soil carbon occurs at a number of time scales, including on the order of minutes (such effects on exudes) or years (effects on litter). In addition, the effects of plant diversity and identity are known to vary both within and over seasons, and this variation is one of the main contributors to biodiversity effects on ecosystem functioning (reviewed in Hooper

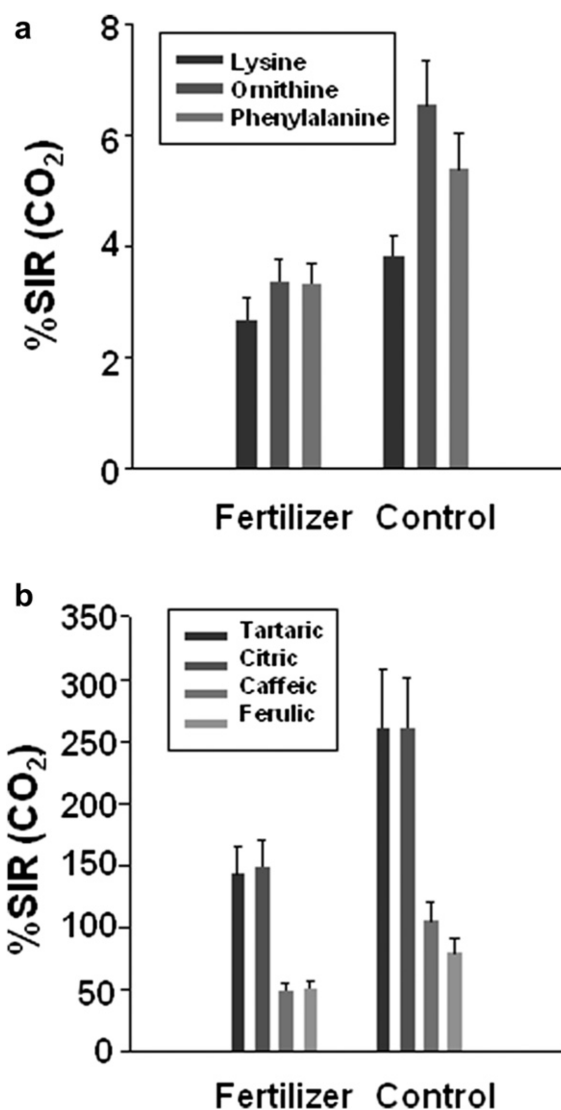

Fig. 3. The effect of 5 years of fertilization ($n = 80$) on mean (+1SE) SIR (%CO_2 required to produce a colour change in the plate well) for (a) amino acids (lysine, ornithine, phenylalanine) and (b) organic acids (tartaric acid, citric acid) and the phenolic acids (caffeic acid, ferulic acid).

et al., 2005). For example, we found effects of removals on soil properties early in this experiment (McLaren and Turkington, 2010) but not in the fifth year when the microbial sampling occurred. Our use of a single sampling date to determine soil microbial structure may have strongly influenced our ability to determine effects of plant functional group composition on the soil microbial community.

Finally, an additional reason for the lack of response by the soil microbial community may be relatively broad criteria of SIR and PLFA to identify microbial groups (Kirk et al., 2004; Leckie, 2005). If microbial changes do occur, but within the broad groups detected by SIR and PLFA, they would not have been detected in this study.

In contrast to the lack of effects of plant functional group identity, fertilization decreased the metabolic diversity of the soil microbial community. SIR showed a decreased ability of the soil microbial community to metabolize amino acids and a phenolic acid. Although Sarathchandra et al. (2001) also reported decreased functional diversity of the microbial community after nitrogen addition, others have reported an increase in SIR values with fertilization (Grayston et al., 2004; Lagomarsino et al., 2007). Our

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decrease could have been caused by an increased dominance of one group of bacteria that thrive under higher nutrient conditions (although the broad groups examined with PLFA showed no change), or an increase in the use of more easily accessible N rather than those tied up in amino acids or could also be the result of increased potassium from fertilization which can cause decreased root exudation (Kraffczyk et al., 1984).

In contrast to the effects of fertilizer, fungicide had no effect on the soil microbial community as measured by either SIR or PLFA, despite increases in soil nitrogen and decreases in mycorrhizal colonization with fungicide application. These results are not consistent with Smith et al. (2000) who reported an increased metabolic activity (SIR) when benomyl was applied to plots for 7 years. Benomyl application also had no impact on total above-ground biomass in our system. The majority of plants at our site may either not be highly dependent on mycorrhizae or they may be slow to respond to benomyl application. Ecosystem properties measured in the first four years of this experiment also detected responses by very few ecosystem properties to benomyl application (McLaren and Turkington, 2010). Surprisingly, despite a decrease in mycorrhizal colonization rates, benomyl had no effect on total fungal biomass, or even arbuscular mycorrhizae fungal biomass (as indicated by C16:1ω5c). Although root colonization decreased substantially, the <10% colonization rate found after fungicide treatments may have been sufficient to maintain mycorrhizal function. As we did not examine species specific colonization rates, we could not determine whether the decrease in colonization was due to a decreases in the colonization of roots of particular species, or across all species which are mycorrhizal in the plant community. A meta-analysis of ectomycorrhizal studies showed little importance of colonization rate in explaining effect size of host growth responses to mycorrhiza (Karst et al., 2008).

5. Conclusion

In the northern Canadian grasslands examined here, plant functional group identity had little influence on the soil microbial community as measured by SIR and PLFA. Direct changes in soil nutrients by fertilization, in contrast, resulted in a decrease in soil microbial metabolic diversity as measured by SIR. We suspect that short-term influences of plant identity on soil, such as plant root exudation, do not have a large impact on the soil microbial community in this system, as this might have resulted in faster responses to plant removals. We suggest that longer-term studies are required to determine if delayed plant influences on soil, such as litter inputs, may result in delayed responses of the soil community to changes in plant identity.

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