Soil microbial community response and recovery following group selection harvest: Temporal patterns from an experimental harvest in a US northern hardwood forest

Tera E. Lewandowski [a,*], Jodi A. Forrester [a], David J. Mladenoff [a], Jennifer L. Stoffel [b], Stith T. Gower [a], Anthony W. D'Amato [c], Teri C. Balser [d,1]

[a] Department of Forest and Wildlife Ecology, University of Wisconsin, Madison, WI 53706, USA
[b] Department of Biology and Chemistry, Upper Iowa University, Fayette, IA 52142, USA
[c] Department of Forest Resources, University of Minnesota, St. Paul, MN 55108, USA
[d] Department of Soil Science, University of Wisconsin, Madison, WI 53706, USA

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ABSTRACT

Forest harvesting and the associated loss of nutrients and carbon has the potential to negatively affect the soil microbial community, which plays a significant role in the health and productivity of the forest ecosystem. We used an experiment to evaluate the effects of group selection using whole-tree harvesting on the soil microbial community in a second growth northern hardwood forest dominated by sugar maple (*Acer saccharum* Marsh.) in northern Wisconsin, USA. We compared the response of the soil microbial community in 200 m^2 and 380 m^2 harvested gaps to unharvested controls during the spring and summer in the first two years post-harvest, and continued to monitor changes in the soil microbial communities and microenvironment in 380 m^2 gaps in years four, five, and seven post-harvest. Changes in community size and composition were assessed using phospholipid fatty acid (PLFA) analysis. We found that the abundance of arbuscular mycorrhizal fungi initially decreased following harvest, while abundance of anaerobic and gram positive bacteria, and the cyclo/pre-cyclo microbial stress ratio increased; responses that can be linked to microclimatic variability and resource accessibility. Neither actinomycete bacteria nor saprotrophic fungi exhibited an initial response to harvest, but in later years, we observed a decrease in actinomycetes and an increase in fungal abundance, suggesting a competitive interaction between the two main complex carbon-utilizing microbial groups. Canopy gap size had a minimal effect on the soil microbial community, resulting in a higher microbial stress ratio in 200 m^2 gaps. The microbial community exhibited seasonal and yearly fluctuation, which reinforces the need for repeated sampling over multiple seasons to correctly interpret management effects. Despite the large amount of seasonal and yearly variability, we began to see signs of recovery in the soil microbial community between two and four years post-harvest. We conclude that group selection accomplished via whole-tree harvesting of this size and scale does not result in long-term effects on the soil microbial community in this temperate northern hardwood forest.

1. Introduction

Soil biota are directly responsible for nutrient availability and soil fertility, which are essential for plant species' growth (Andren and Balandreau, 1999; McGuire and Treseder, 2010). A complex soil microbial community (SMC) will enhance the decomposition of diverse organic carbon (C) sources and subsequent mineralization to CO_2 via heterotrophic respiration, or stabilization of C in the soil via microbe-derived molecules (Chabbi and Rumpel, 2009; McGuire and Treseder, 2010; Six et al., 2006). Changes in aboveground vegetation (Wardle et al., 2004; Zak et al., 2003), soil microclimate (Boot, 2011; Schimel et al., 2007; Zogg et al., 1997), C availability (Fierer et al., 2003), and nutrient inputs (Pennanen et al., 1999) can mediate changes in the belowground SMC; all of which can be affected by forest harvesting. Most research on the

response of the SMC to forest harvesting focus on large, intensive harvests (Table 1), and far fewer focus on alternative management strategies such as traditional group selection harvesting, which is a forest management technique where specific age or bole-diameter classes of trees are harvested in concentrated areas, creating a mosaic of variable-sized canopy gaps across the forested landscape. In the Great Lakes region, USA, group selection harvesting is widely implemented in sugar maple (*Acer saccharum* Marsh.) dominated stands (Crow et al., 1981). Therefore, it is important to understand how group selection harvesting activities influence the SMC due to their significant role within the forest ecosystem.

Naturally occurring forest canopy gaps, ranging in size from 10 s to 100 s m^2, are important structural components of northern temperate forest ecosystems in the Great Lakes region (Dahir and Lorimer, 1996; Frelich and Lorimer, 1991; Goodburn and Lorimer, 1999), resulting in spatial and temporal variability across the forested landscape (Frelich et al., 1993). The creation of natural forest gaps has important consequences for aboveground species composition and age structure (Lorimer, 1989), microclimatic variables such as incoming solar radiation, soil moisture and soil temperature (Scharenbroch and Bockheim, 2007), soil nitrogen dynamics (Mladenoff, 1987; Scharenbroch and Bockheim, 2007; Schliemann and Bockheim, 2014), fine-root growth (Bauhus and

Bartsch, 1996), and the quantity and quality of above and belowground organic matter inputs to the soil system. Research on the effects of natural canopy gaps on the SMC in temperate regions has found that gaps can lead to increases in microbial biomass in mixed northern hardwood–hemlock (*Tsuga canadensis* L.) forests (Scharenbroch and Bockheim, 2007), and reduced ectomycorrhizal fungi (EMF) in coniferous forests (Griffiths et al., 2010). In addition, gap size (ranging from 27 to 590 m^2) can affect the SMC composition, with larger gaps leading to reduced microbial biomass and arbuscular mycorrhizal fungi than smaller gaps (Schliemann and Bockheim, 2014). Canopy gaps resulting from group selection harvesting differ from natural canopy gaps due to potential soil compaction effects from harvesting equipment, and a significant reduction in residual aboveground coarse and fine woody debris.

While forest harvesting can lead to a reduction in microbial biomass, the response of the SMC composition may be a more sensitive indicator of environmental changes post-harvest (Siira-Pietikäinen et al., 2001). Phospholipid fatty acid (PLFA) analysis has proven to be a useful tool for measuring both microbial biomass and compositional changes in the SMC in response to ecosystem disturbance (Hynes and Germida, 2012; Ramsey et al., 2006) because PLFAs are found in every living cell and degrade relatively quickly following cell death (Frostegård et al., 2011). Following a

Table 1
Summary of research utilizing PLFA analysis to determine the effects of forest harvesting on the SMC in mineral soil. GmP = gram positive; GmN = gram negative; WTH = whole tree harvest; BOH = bole only harvest; GTR = green-tree retention; FFR = forest floor removal; N.D. = no data. In general, intensive clearcut harvesting has a greater effect on SMC than less intensive treatments such as thinning.

Species (location)	Overstory treatment/harvest method	Harvest size	Years post-harvest	Microbial biomass	EMF + Saprotrophic (fungi)/AMF	Bacteria/community structure
[1]Beech (Germany)	Thinning 37–75%/BOH	0.53 ha	1[*]	Variable response	No change	No change
[2]Norway spruce (Finland)	Thinning 30%/WTH[*]	1.0 ha	1–2	No change	No change	No change
[3]Pine (Germany)	Thinning 20–60%/WTH[*]	0.13 ha	5	No change	No change	No change
[2]Norway spruce (Finland)	Clearcut/WTH[*]	1.0 ha	1–2	Decreased	Decreased fungal, increased AMF abundance	Abundance of some GmP, GmN, and actinomycete markers increased.
[4]Lodgepole pine (Canada)	Clearcut/BOH[*]-rake and burn	9 ha	2–3	Decreased in year 2	No data	Community structure shifted through time
[5]Lodgepole pine (Canada)	Clearcut/BOH[*]-rake and burn	2–49 ha	1–19	No change	Increased fungal biomass through time	Community structure shifted through time
[6]Oak/Hickory (Missouri)	Clearcut/BOH, WTH+ FFR	0.4 ha[*+]	4	No change	Increased AMF abundance in BOH	Abundance of saturated lipids increased, CYC ratio decreased; abundance of GmN increased in BOH
[7]Aspen, Spruce (Canada)	Clearcut/WTH, 20% GTR, 50% GTR	10 ha	4–5	No Change	Increased AMF abundance in WTH, 50% SR	No change
[8]Mixed conifer (California)	Clearcut/BOH, WTH+ FFR	0.4 ha[*+]	6	Decreased	Decreased fungal biomass in BOH	No change
[8]Loblolly pine (N. Carolina, Louisiana)	Clearcut/BOH, WTH+ FFR	0.4 ha[*+]	8, 10	No change	No change	No change
[9]Aspen (Michigan)	Clearcut/BOH, WTH, WTH+ FFR	0.25 ha	8, 10	Decreased	Decreased fungal abundance	No change
[10]Douglas-fir (Washington)	Clearcut/N.D.	Landscape scale[*]	8, 25	Decreased	Increased fungal abundance (25 years)	Abundance of GmP and actinomycetes increased (8 years)
[11]Mixed conifer (Montana)	Clearcut/N.D	N.D.	45	Decreased	Decreased fungal, increased AMF abundance	Abundance GmN decreased, GmP increased.
[12]Ponderosa pine (Wyoming)	Various/N.D.	>1.0 ha[*]	Various 45–90	No change	Decreased fungal biomass	Biomass GmP decreased, GmN increased.
[12]Lodgepole pine (Wyoming)	Clearcut/N.D	>1.0 ha[*]	45	Decreased	Decreased fungal, AMF biomass	Biomass GmP, GmN decreased.

(1) Grayston and Rennenberg (2006), (2) Siira-Pietikäinen et al. (2001), (3) Maassen et al. (2006), (4) Hynes and Germida, 2013, (5) Hynes and Germida (2012), (6) Ponder and Tadros (2002), (7) Hannam et al. (2006), (8) Busse et al. (2006), (9) Hassett and Zak (2005), (10) Moore-Kucera and Dick (2008), (11) Mummey et al. (2010), (12) Chatterjee et al. (2008).

[*] Harvest methods not clear; this a best guess of harvest type/age.
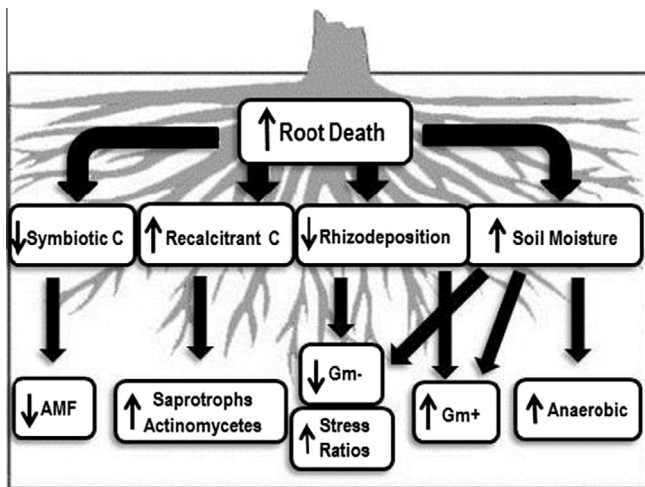[*+] Harvest methods not clear; data from Powers et al. (2005).

Fig. 1. Conceptual diagram of canopy gap creation effects and hypothesized responses of the SMC in a sugar maple dominated forest.

forest harvest, in general, we would expect the SMC to change in predictable ways due to effects on the soil microclimate, and changes in C and nutrient allocation (Fig. 1). However, when we compare the results from multiple harvesting studies, we see that the actual response of the SMC is highly variable, most likely due to differences in the tree species harvested, overstory treatment, harvest method, amount of organic biomass retained post-harvest, harvest size, time since disturbance, high degree of spatial variability in harvest effects, and geographic region and associated climate (see Table 1). Therefore, while it is important to conceptually understand how the SMC might respond to a harvesting disturbance, it is also important to account for and acknowledge these aforementioned differences when making comparisons among multiple harvesting studies.

When a tree is harvested, photosynthesis and thus belowground C allocation stop, leading to reduced abundance of the mycorrhizal fungi associated with the harvested tree species (Smith and Read, 2008) (Fig. 1). Forest harvesting also leads to root death, resulting in an increase in complex plant structural, or recalcitrant, C. Because saprotrophic fungi (Wolf and Wagner, 2005) and actinomycete bacteria (Deslippe et al., 2012; Nakatsu, 2005) are able to utilize recalcitrant C sources, both groups would be expected to increase in abundance following forest harvest (Fig. 1). Finally, harvesting reduces the availability of labile C inputs to the soil through rhizodeposition (Hütsch et al., 2002), and increases soil moisture due to less plant uptake (Schatz et al., 2012; Stoffel et al., 2010). Nutrient limitation and moisture stress negatively affect gram negative bacteria and lead to an increase in microbial stress ratios (Guckert et al., 1986; Kieft et al., 1997), but have less of an effect on more resistant gram positive bacteria (Deslippe et al., 2012; Fierer et al., 2007; Ringelberg et al., 2008; Treonis et al., 2004). Additionally, higher soil moisture would result in higher anaerobic soil bacterial abundance (Fig. 1).

Seasonal variability has a large influence on the SMC composition (Leckie, 2005) and research has shown that time periods with increased moisture are correlated with higher bacterial abundance (Myers et al., 2001), while drier periods lead to reduced microbial biomass and increased stress ratios (Moore-Kucera and Dick, 2008). We expect that microbes with a filamentous growth form, including fungi and actinomycetes, will better tolerate moisture deficiency and proliferate during the warm, dry summers. Single celled microbes will decrease in abundance, while their associated

stress ratios will increase during high moisture stress periods (Schimel et al., 2007).

In this research, we used an experimental approach to characterize the response of the SMC following a group selection harvest in a second-growth, sugar maple dominated forest in northern Wisconsin, USA. We used PLFA analysis to assess the response of microbial biomass, community structure, specific microbial guilds and microbial stress. Our primary objective in this experiment was to determine the magnitude and duration of the SMC response to group selection harvesting. We hypothesized that increased soil moisture and changes in C dynamics post-harvest would result in decreased AMF and gram negative bacterial abundance, and an increase in the abundance of saprotrophic fungi, actinomycetes, gram positive bacteria, anaerobic bacteria, and microbial stress ratios when compared with unharvested controls (Fig. 1). By analyzing the SMC response during two seasons (spring and summer) and five years, we were also able to characterize the response of the SMC to climatic variation.

2. Materials and methods

2.1. Site description

This study was part of a large, long-term manipulative experiment that was implemented to quantify the effect of whole-tree removal and amount of coarse woody debris retained on biodiversity and ecosystem function in a northern hardwood forest (Dyer et al., 2010; Forrester et al., 2013,2012; Schatz et al., 2012; Stoffel et al., 2010). The 300 ha field site is located in the southernmost portion of the Flambeau River State Forest, Rusk County, north-central Wisconsin (43° 37.4N, 90° 47.8W). Soils are classified as silt loams (Glossudalfs) of the Magnor (somewhat poorly drained) and Freeon (somewhat well drained) series overlaying dense glacial till. Average air temperature is 5.9 °C, with a mean annual precipitation of 33 in (1971–2000). The growing season median length is 105 days (1971–2000; Midwest Regional Climate Center). The stand can be described as an even-aged, second-growth forest that was logged in the early 20th century. Presently, the dominant tree species is sugar maple, followed by American basswood (*Tilia americana* L.), and white ash (*Fraxinus americana* L.). Historically, the original logged forest was dominated by old and mature eastern hemlock, yellow birch (*Betula alleghaniensis* Britton), and sugar maple (Schulte et al., 2002). Burton et al. (2011, 2014) presents a detailed description of plant communities within the site.

2.2. Experimental design

We used three experimental treatments to address our objectives: group selection whole-tree harvested gap; mechanized (disturbed with equipment, but unharvested) control; and a true control (no equipment present, no harvest). Treatments were randomly assigned to five replicate 80 × 80 m whole plots. The gap addition treatment is a split-plot that includes three variable sized subplots; only two of which were sampled in this study (medium and large). Within each harvested plot, trees were removed to create either a 200 m^2 (16 m diameter) or 380 m^2 (22 m diameter) canopy opening, referred to as medium and large canopy gaps, respectively. These canopy gap sizes are within the range of natural canopy gaps in old-growth northern hardwood forests in the Great Lakes region (Dahir and Lorimer, 1996). Treatments were implemented during winter (January) 2007 under snow and frozen ground conditions using a PONSSE Ergo harvester and PONSSE Buffalo forwarder (Ponsse, 14 Ponsse Oyj, Vieremä, Finland) to reduce soil compaction effects.

2.3. Soil microbe sampling

Soil samples were collected in spring (May) and summer (August) of 2007, 2008, 2010, 2011 and 2013, which represent growing seasons 1, 2, 4, 5 and 7 post-treatment, respectively. Two north–south transects (12 m and 8 m in length within large and medium gaps, respectively) were established 4 m apart in the center of each subplot. Sampling points were located at 4 m intervals along each transect, resulting in 6 and 8 sampling locations per subplot (Supplementary Fig. 1). In years 1 and 2, soil was sampled in half of the locations (randomly determined) in 4 of the 5 plots/treatment. During year 1, all 3 treatments were sampled for a total of 168 samples (i.e. 2 seasons × 3 treatments × 4 plots × 7 subsamples from 2 subplots); in year 2 only control and harvest treatments were sampled for a total of 112 samples (Stoffel, 2009). In years 4, 5, and 7, soil was sampled in 3 locations (the 2 northern, and the most southern) in 5 plots (large gaps only), for a total of 60 samples per year (i.e. 2 seasons × 2 treatments × 5 plots × 3 subsamples from large subplot). Mineral soil was collected using a 2.36 cm push probe (Hoffer sampler, JBK, Beavercreek, OH) to a depth of 15 cm. Samples were composites of either 3 (years 1–2) or 2 (years 4, 5, 7) cores per location. Soil temperature was recorded at a depth of 10 cm in years 1 and 2, and 7.5 cm in years 4, 5, and 7 using a portable probe digital long stem thermometer (Model No. 15-078k, Fisher Scientific). Soil moisture was measured to a depth of 6 cm at the time of soil collection with a calibrated TDR probe attached to a HH2 Moisture Meter (Delta-T Devices, Cambridge, England).

Soil samples were stored at 5 °C the day of collection, and transported to the University of Wisconsin-Madison within 4 days where they were frozen at −20 °C. Frozen samples were lyophilized (Freezemobile 12, Virtis of Gardiner, NY), roots and stones were removed, and samples were ground in preparation for microbial lipid extraction.

2.4. Lipid extraction and analysis

We used PLFA analysis to characterize the SMC. By extracting lipid biomarkers from microbial cell membranes, we can make inferences about general SMC structure (White and Ringelberg, 1998). Lipids were extracted using a modified PLFA and fatty acid methyl ester (FAME) method (Balser and Firestone, 2005). Throughout the procedure, Teflon tubes and caps were hexane rinsed, and all glassware was baked at 550 °C for 3 h to sterilize and remove exogenous lipids. Briefly, membrane lipids were extracted from 3.5 g of lyophilized soil in a two-phase aqueous-organic extraction, using a 2:2:1 ratio of 0.1 M phosphate buffer (pH 7.5), methanol, and chloroform (Bligh and Dyer, 1959). Samples were extracted twice using this method, and after a phase-separation the organic phase was isolated and dried down using a RapidVap (LabConco, Kansas City, MO). Finally, lipids were saponified, subjected to alkaline methanolysis, and isolated in a hexane extraction.

A 2 µl injection of the FAMEs from the extracted lipids were analyzed using a Hewlett-Packard 6890 Gas Chromatograph (San Fernando, CA) with a flame ionization detector configured and maintained for lipid analysis according to the recommendations of MIDI (MIDI Inc., Newark DE). The peaks on the resulting chromatogram were identified by comparing retention times to straight-chain, FAME calibration standards using the MIDI Sherlock microbial identification system (MIS) software (MIDI Inc., Newark DE). To quantify the amount of individual lipids, peak area was first multiplied by a response factor (Rfact) derived from running the MIDI calibration standard and included in the Sherlock MIS report. This response factor corrects for differences in detector response across the range of chain-lengths (Christie, 1989), normalizing the peak area to lipid mass relationship (Personal communication, MIDI Inc., Newark, DE). Rfact corrected sample peak area was then quantified by comparison with the Rfact corrected peak area of two external standards, methyl nonanoate (9:0) and methyl nonadecanoate (19:0), of known concentration.

2.5. Lipid data processing

Lipid data were processed using an open source licensed Microsoft Access® Database developed and designed by Dr. Devin Wixon at UW-Madison (Devin Wixon, 2013, Lipid GC Process). The absolute abundance of lipids is expressed as µmol lipid/g soil. Summed

Table 2
Descriptions and phospholipid fatty acids (PLFAs) included in the 6 microbial guilds and 2 stress ratios used as indicators of the SMC response to canopy gap creation.

Taxonomic group	Description	PLFAs included	References
Gram-positive bacteria	Common, single-celled, slow growing soil bacteria with a thick cell wall that increases their resilience to physical stress, such as turgor pressure. Able to use more complex and older SOM than GmN	i14:0, i15:0, a15:0, i16:0, i17:0, a17:0	(1, 2, 4, 12, 13, 14)
Gram-negative bacteria	Common, single-celled soil bacteria with a thin cell wall, resulting in a higher susceptibility to physical stresses. Use easily degradable, recent plant carbon sources	16:1 ω7c, 17:0cyc, 18:1 ω7c, 19:0cyc, 19:0cyc 11-12 2OH	(1,3,4,6,12, 14)
Actinomycete bacteria	Slow-growing, hyphal, GmP bacteria that are able to break down a large variety of organic compounds	16:0 10 methyl, 17:0 10 methyl, 18:0 10 methyl	(5,6,7,12)
Saprotrophic fungi	Important in breaking down complex organic compounds such as chitin, cellulose, hemicellulose, and lignin	18:2ω6, 9c	(5,6,7,8, 17,19)
Arbuscular mycorrhizal fungi	Symbiotic soil fungi that associate with herbaceous and woody plants, acquiring and assimilating nutrients for their symbionts and increasing soil aggregate stability	16:1ω5c	(9,15,16)
Anaerobic bacteria	Bacteria that thrive in low oxygen environments	19:0cyc, 17:0cyc,19:0cyc 11-12 2OH	(3,18)
Stress ratios		**PLFAs included**	**References**
CYC	GmN bacteria experiencing resource stress alter their monoenoic fatty acids to cyclopropyl, resulting in an increased CYC ratio	17:0cyc, 19:0cyc/16:1ω7c, 18:1ω7c	(5, 10, 11)
SAT	GmN bacteria experiencing resource stress increase cell membrane fluidity by altering unsaturated fatty acids to saturated, resulting in an increased SAT ratio	14:0, 15:0, 16:0, 18:0/16:1ω5c, 16:1ω7c, 16:1ω9c, 17:1ω7c, 18:1ω7c, 18:1ω9c	(5, 10, 11)

(1) Ringelberg et al. (1997), (2) Zelles et al. (1992), (3) Wilkinson (1988), (4) Waldrop et al. (2000), (5) Bossio et al. (1998), (6) Zelles (1999), (7) Frostegård et al. (1993), (8) Frostegård et al. (1996), (9) Olsson (1999), (10) Guckert et al. (1986), (11) Kieft et al. (1997), (12) Nakatsu (2005), (13) Amelung et al. (2008), (14) Kramer and Gleixner (2008), (15) Sylvia (2005), (16) Wilson et al. (2009), (17) Morton (2005), (18) Vestal and White (1989), (19) Wolf and Wagner (2005).

absolute abundances over all lipids was used as an index of microbial biomass (Balser and Firestone, 2005; Hill et al., 1993; White et al., 1979; Zelles et al., 1992). The relative amount of individual lipids was determined by calculating relative mol% (moles of lipid/total moles lipid in sample) and used for microbial community composition analyses. Lipids with an average relative abundance of less than 0.5 mol% were discarded from analysis, resulting in a multivariate dataset that included 30 relative mol% lipids. Fatty acid nomenclature is as described elsewhere (Frostegård and Bååth, 1996; Zelles, 1997; Aanderud et al., 2008). PLFAs were categorized into 6 functional groups, or "guilds", that can be used to describe different portions of the SMC; including, gram positive bacteria (GmP), gram negative bacteria (GmN), actinomycete bacteria (Act), saprotrophic fungi (fungi), arbuscular mycorrhizal fungi (AMF), and anaerobic bacteria (Table 2). The saprotrophic fungal lipid could be an indicator of both EMF and saprotrophic fungi; however, because there are few EMF tree species in our site, we assume that most, if not all, of the fungi in this group are actually saprotrophic, and refer to this group accordingly. Additionally, it should be noted that some indicators are also found in small amounts in other microbial groups, particularly 16:1ω5c (AMF), which is also GmN bacterial. Finally, the ratio of saturated to monounsaturated PLFAs (SAT) and the ratio of cyclopropyl to monoenoic precursors (CYC) are presented as indicators of microbial stress (Table 2).

2.6. Statistical analyses

We used generalized linear mixed models to evaluate the effects of the experimental treatments, subplot size, season, and year on individual soil microbe guilds and stress ratios, total microbial biomass, and soil temperature and moisture. Mixed model ANOVAs were performed using proc mixed in SAS version 9.3 (SAS, 2010; System for Windows, SAS Institute Inc., Cary, NC, USA). Sampling locations were averaged to the subplot level prior to analysis. Initially, to test for soil compaction effects during the first year, we compared mechanical control and control treatments using treatment, subplot size, and season as fixed effects. To analyze the effects of harvesting, the temporal response was split into two separate time periods due to moderate changes in sampling design. For the early treatment response (years 1–2), we tested the fixed effects of treatment, subplot size, season, and year. For the mid-term response (years 4, 5, 7) we tested the fixed effects of treatment, season, and year. In all ANOVA designs, replicate plots within treatments were analyzed as a random effect.

Multivariate analyses were performed using PRIMER version 6 (Clarke and Gorley, 2006) and the PERMANOVA + add-on package (Anderson et al., 2008). Multivariate variability was analyzed using Permanova and principal coordinates analysis (PCO) within the PERMANOVA + add-on package. Permanova, or distance-based permutational MANOVA (Anderson, 2001), is a statistical routine that tests the multivariate response to treatment factors in an ANOVA design using any resemblance measure (Anderson et al., 2008). PCO analysis, which is an unconstrained ordination of multivariate data, was used to aid in visualization of the data in multivariate space (Anderson et al., 2008; Clarke and Gorley, 2006; Gower, 1966).

We used data from large and medium subplots in years 1 and 2, and large subplots in years 4, 5, and 7 to assess control and harvest treatment differences within sampling periods, and seasonal variation through time. Multivariate variability was analyzed at each level of nesting (entire dataset, individual years, sampling period), using the Permanova routine. Permanova analyses were performed using a Bray–Curtis resemblance measure with 9999 random permutations, type III sum of squares, and permutation of residuals under a reduced model. Distances among treatment group centroids within sampling periods were calculated based on principal coordinates and visualized using PCO (Clarke and Gorley, 2006) with Pearson correlation vector overlays ($r > 0.2$) of microbial guilds, soil temperature and moisture to better understand seasonal multivariate patterns.

3. Results

3.1. Soil microenvironment- annual, seasonal and treatment influences

Both soil moisture and temperature during early and mid-term response periods indicated highly significant variation by year and

Table 3
ANOVA p-values ($p < 0.1$) for main effects of treatment (unharvested control and harvested gap), subplot size (large and medium), year (early response = years 1 and 2; mid-term response = years 4, 5, and 7), and season (spring and summer) and their interactions on the soil microenvironment and SMC variables. Dashes indicate non-significant effects. Treatment = Treat; Subplot size = Sub; Year = Ye; Season = Seas.

	Moisture	Temp.	Biomass	AMF	Anaerobe	Act	Fungi	GmP	GmN	CYC	SAT
Early response period											
Treatment	–	0.0008	–	0.01	0.02	–	–	–	–	0.06	–
Subplot size	–	–	0.09	–	–	–	–	–	–	–	0.04
Treat * Sub	–	–	–	–	–	–	–	–	–	–	–
Year	0.0001	0.0001	–	0.0001	0.0001	0.0001	0.004	0.0001	0.0001	–	0.0001
Treat * Ye	–	0.03	–	0.004	–	–	–	–	–	–	–
Ye * Sub	–	–	–	–	–	–	–	–	–	–	0.09
Treat * Ye * Sub	–	–	–	–	–	–	–	–	–	–	–
Season	0.0001	0.0001	–	0.0001	0.09	–	0.01	–	0.06	–	0.0001
Treat * Seas	–	0.0001	–	0.0001	0.008	0.02	–	–	0.08	0.005	–
Sub * Seas	–	0.02	–	–	–	–	–	–	–	–	–
Treat * Sub * Seas	–	–	–	–	–	0.02	–	0.05	–	–	–
Ye * Seas	0.0001	0.0001	0.01	0.0001	0.01	–	0.02	–	0.02	0.0004	0.0006
Treat * Ye * Seas	0.04	–	0.08	0.0001	0.06	–	–	–	–	–	0.0005
Ye * Sub * Seas	–	0.09	–	–	–	–	0.03	–	–	0.01	–
Treat * Ye * Sub * Seas	–	–	–	–	0.09	–	–	–	–	0.03	–
Mid-term response period											
Treatment	–	0.002	–	–	–	–	–	–	–	–	–
Year	0.0001	0.0001	0.0001	0.03	0.0002	0.0001	0.0002	0.002	0.0001	–	0.01
Treat * Ye	–	–	–	–	–	–	–	–	–	–	–
Season	0.0001	0.0001	–	0.001	–	–	0.04	–	0.03	0.03	0.01
Treat * Seas	–	0.015	–	0.05	–	–	–	–	–	–	–
Ye * Seas	0.0001	0.0001	0.001	0.04	–	–	0.06	0.09	–	0.08	–
Treat * Ye * Seas	–	–	–	–	–	–	–	–	–	–	–

season (Table 3; Fig. 3), which paralleled patterns of variation in air temperature and precipitation (Fig. 2). Summer sampling periods were generally drier than spring, with the exception of year 4, which also had higher than average summer precipitation. Dry soils in controls during the summer of years 1 and 2, and in both treatments during year 7 corresponded with less than average summer precipitation (Figs. 2 and 3). Soil temperatures during the spring were generally less than 14 °C, except for during year 4, which also had the highest spring air temperature of all sampling years. Summer soil temperature was lowest during year 7 at 15 °C (Figs. 2 and 3).

The temporal responses of the soil microenvironmental parameters to the experimental treatments varied. Soil temperature and moisture did not differ between mechanical control and unharvested control treatments during the first year ($p > 0.1$; data not shown). Harvested gap soils were wetter than unharvested controls during the first two post-harvest growing seasons (Fig. 3a). Soil in gaps was also warmer than controls during the first spring post-harvest (Fig. 3b). Soil temperature and moisture did not differ between large and medium harvested gaps during the first two years ($p > 0.1$; data not shown). In the mid-term sampling period, soil continued to be significantly warmer in gaps during spring sampling periods (Fig. 3b), but soil moisture no longer differed between treatments (Fig. 3a).

3.2. Microbial biomass – annual, seasonal and treatment influences

Annual and seasonal differences in temperature and moisture (Table 3, Figs. 2 and 3) corresponded with the patterns of variation in soil microbial biomass that we measured throughout the experiment (Supplementary Fig. 2). We measured the lowest microbial biomass in year 7 when precipitation and air temperature were below average. Biomass was highest in spring of year 4 and summer of year 5 when temperatures were above average with moderate precipitation. Forest harvesting did not affect overall soil microbial biomass, and we did not observe any seasonal or yearly trends in total microbial biomass through time (Table 3; Supplementary Fig. 2).

3.3. Early SMC response – years 1 and 2

The relative abundance of microbial guilds varied minimally between mechanical control and unharvested control treatments during the first year post-harvest. During the first spring, both anaerobic bacteria (mechanical = 7.9, control = 7.0; $p = 0.04$) and the CYC stress ratio (mechanical = 0.53, control = 0.45; $p = 0.04$) were more abundant in mechanical than control treatments. No other significant differences between these two treatments were found (data not shown).

All of the microbial guilds and the SAT stress ratio showed significant variation by year, during the early response periods (Table 3). AMF, fungi, GmN bacteria, and the SAT stress ratio also showed significant, discernible patterns in seasonal variation as well; AMF, fungi, and the SAT ratio were generally higher in the summer, while GmN were generally higher in the spring (Table 3; Figs. 4 and 5). Forest harvesting decreased the abundance of AMF in canopy gap soils compared with controls; a result that was more significant during the summer sampling periods than the spring
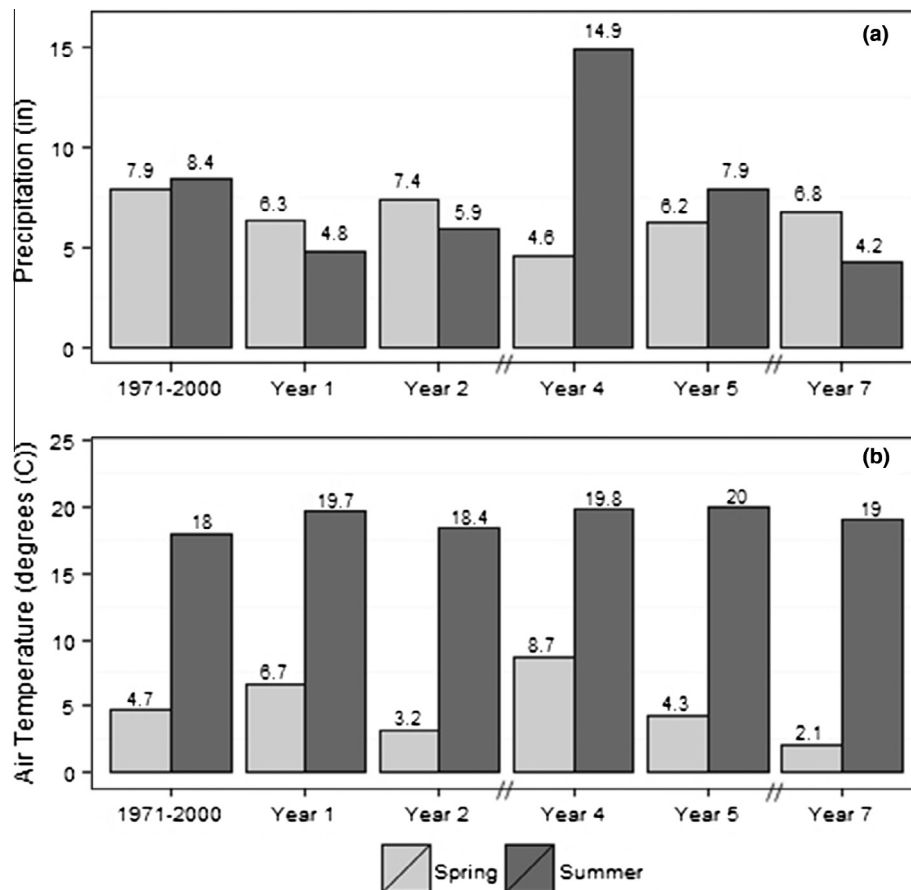
Fig. 2. Total precipitation (a) and average temperature (b) during the spring (March–May) and summer (June–July) of the 5 sampling years, and the average of a preceding 30 year time interval (1971–2000).
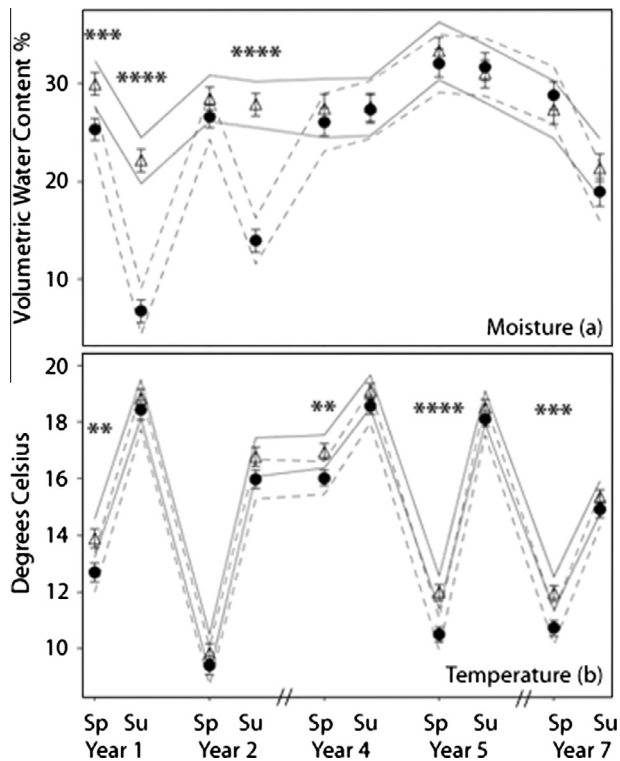
Fig. 3. Gap (open triangles) and control (closed circles) treatment means for soil moisture (6 cm) and temperature (7.5 cm) in spring (Sp) and summer (Su) of each sampling year. Sample sizes are $n = 4$ for years 1 and 2, and $n = 5$ for years 4, 5, 7. Means are graphed with standard error bars and 95% confidence intervals (gap = solid lines, and control = dotted lines). Asterisks represent pairwise significance levels (*$p < 0.1$; **$p < 0.05$; ***$p < 0.01$; ****$p < 0.001$).

(Fig. 4a). The large increase in AMF abundance in controls during the summer of year 1 corresponded with a decrease in soil moisture (Fig. 3). Anaerobic bacteria in harvested gaps increased in abundance during the summer of years 1 and 2 when compared with controls, but were not different from controls during the spring sampling periods (Fig. 4b). GmP bacteria were more abundant in gap soils than unharvested controls during all four sampling periods; significantly so during the spring of year 1 (Fig. 4e). Actinomycetes, fungi, and GmN bacteria did not exhibit a significant response to treatment in the first two years following harvest (Table 3; Figs. 4c, d, f). The CYC stress ratio was higher in harvested gaps than controls, significantly so from the summer of year 1 through the summer of year 2 (Fig. 5a). The SAT stress ratio exhibited a mixed response, and was not significantly different between gap or control treatments during any of the sampling periods (Table 3; Fig. 5b).

Subplot size had limited effects on the SMC. The SAT stress ratio was significantly different by subplot size; a difference driven by a higher ratio in medium harvest gaps compared with large harvest gaps during the spring (medium = 0.76, large = 0.66; $p = 0.006$) and summer (medium = 0.81, large = 0.73; $p = 0.02$) of year 2 (Table 3).

3.4. Mid-term SMC response— years 4, 5, and 7

In the mid-term response period, all of the microbial guilds and the SAT stress ratio showed significant variation by year (Table 3). AMF continued to be more abundant during the summer, while fungi and GmN bacteria had a mixed seasonal pattern in years 4, 5, and 7. Both the SAT and CYC ratio were higher during the summer with the exception of year 7, when spring and summer ratios were more similar (Table 3; Figs. 4 and 5). Few functional or stress ratio differences due to treatments were found. AMF abundance

remained lower in gaps relative to controls in the fourth year post-treatment but with time became more similar (Fig. 4a). Additionally, actinomycetes decreased (Fig. 4c) and fungi increased (Fig. 4d) within harvested gaps, especially during the spring of year 7. Gram positive, GmN, anaerobic bacteria, CYC, and SAT response indicators all showed mixed, non-significant responses to harvesting during the mid-term response period (Table 3; Figs. 4 and 5).

3.5. SMC composition – annual, seasonal, and treatment influence

Multivariate Permanova analyses indicate that the SMC varied significantly by year ($p = 0.0001$) and season ($p = 0.0001$) (Fig. 6). The SMC composition during year 1 separated from other sampling years along PCO axis 1, which was highly positively correlated with the relative abundance of soil bacteria ($r = 0.86–0.94$) and the CYC stress ratio ($r = 0.59$). In year 7, the SMC composition separated from the other sampling years along PCO axis 2, which was positively correlated with AMF ($r = 0.39$) and fungal relative abundance ($r = 0.85$) (Fig. 6). Harvest and control treatments did not differ during the spring sampling periods (Permanova, $p > 0.1$); therefore, we only present PCO analysis from the summer of all sampling years (Fig. 7a–e). In the summer of year 1, treatments were significantly different (Permanova, $p = 0.0008$), but not subplot size, or the interaction. The SMC in harvested gaps and controls primarily separated along PCO axis 1, which was positively correlated with AMF relative abundance ($r = 0.93$), and negatively correlated with anaerobic bacterial relative abundance ($r = -0.62$), bacterial stress ($r = -0.61$ to -0.75), and soil moisture ($r = -0.75$) (Fig. 7a). During the second summer, both treatment (Permanova, $p = 0.0788$) and large and medium harvested gaps differed significantly (Permanova, $p = 0.08$). The SMC composition in controls separated from gaps primarily along PCO axis 2 and was positively correlated with AMF ($r = 0.79$) and fungal ($r = 0.57$) relative abundance, while the SMC composition in gaps was most correlated with bacterial stress ratios ($r = -0.43$ to -0.52), anaerobic bacterial relative abundance ($r = -0.42$), and soil moisture ($r = -0.49$) (Fig. 7b). Permanova analysis indicated no treatment differences in years 4 ($p = 0.2$), 5 ($p = 0.6$), or 7 ($p = 0.4$) (Fig. 7c–e). In general, bacterial Pearson correlation vectors grouped well and were associated with high moisture, while AMF and fungal markers tended to be located opposite the soil moisture vector, indicating a negative relationship with soil moisture.

4. Discussion

4.1. Recovery of the SMC following harvest

Harvesting had no consistent effect on total microbial biomass, but did initially alter the composition of the SMC during the first two years post-harvest. The SMC in gaps were distinguished by low AMF abundance, and high abundances of bacterial guilds and stress ratios. However, these differences largely disappeared by year 4, indicating the SMC recovered from group selection harvesting between 2 and 4 years post-harvest. This recovery time is quicker than most large clearcut harvests, which can have treatment effects for much longer timespans (Table 1).

The relatively quick recovery of SMC structure may be attributed to both the small size and minimal soil disturbance of the harvest. Additionally, rapid regeneration of vegetation within the harvested gaps reduces soil moisture and increases AMF hosts. Gap size has been shown to influence the SMC, most likely due to changes in C availability (Schliemann and Bockheim, 2014); however, in this study the SMC within the two experimental harvest sizes were largely similar. In a separate study, Stoffel et al. (2010) found that total soil CO_2 respiration, which includes both
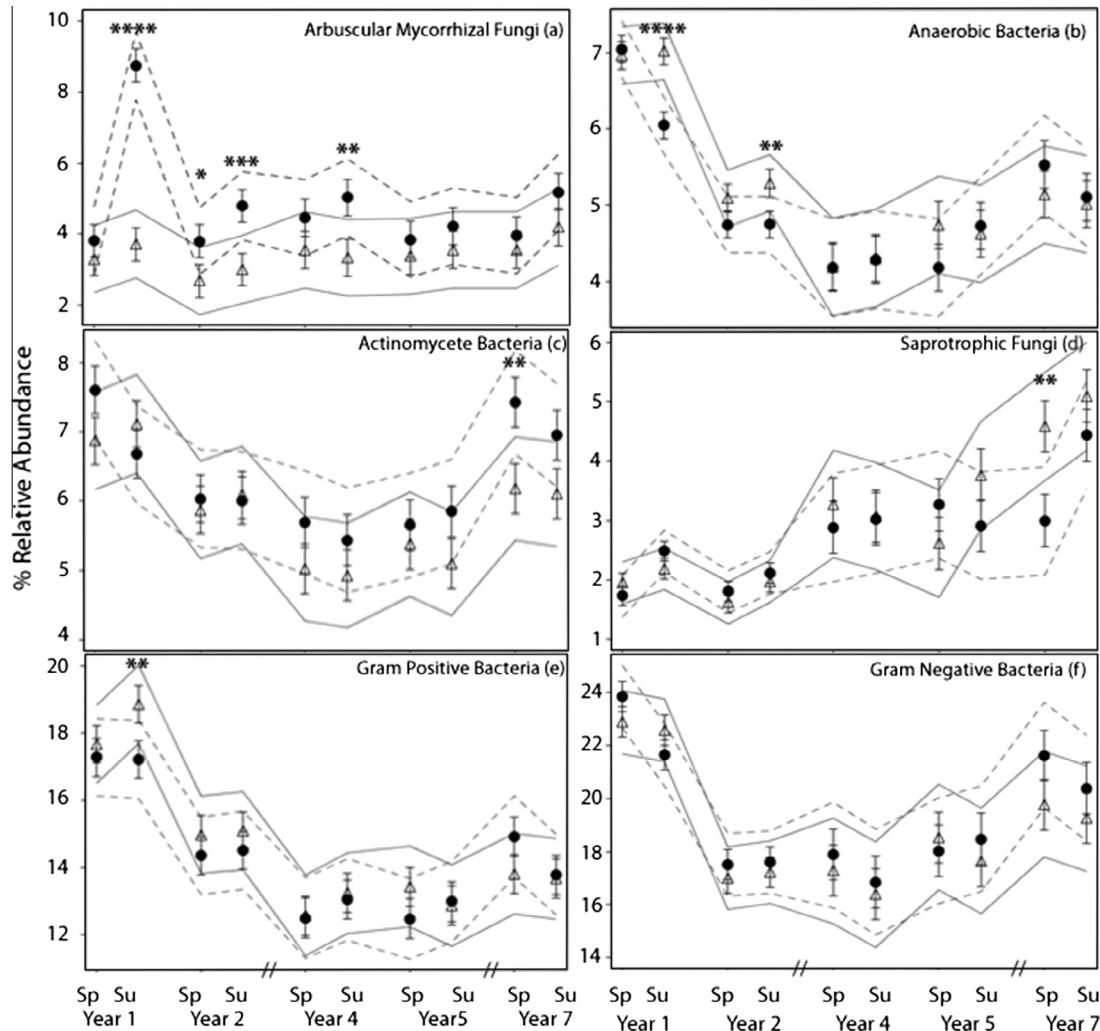
Fig. 4. Gap (open triangles) and control (closed circles) treatment means for the % relative abundance of microbial guilds in spring (Sp) and summer (Su) of each sampling year. Sample sizes are $n = 4$ for years 1 and 2, and $n = 5$ for years 4, 5, 7. Means are graphed with standard error bars and 95% confidence intervals (gap = solid lines, and control = dotted lines). Asterisks represent pairwise significance levels (*$p < 0.1$; **$p < 0.05$; ***$p < 0.01$; ****$p < 0.001$).

heterotrophic and autotrophic sources, did not differ between large and medium canopy gaps either. These sizes may not differ functionally, whereas had we included either single-tree selection harvests or clearcuts, we may have seen more variation in the SMC due to harvest size (Griffiths et al., 2010; Zak, 1998). Additionally, the minimal impact of the harvesting equipment on soil compaction may have contributed to the quick recovery of the SMC. Soil compaction can have a large effect on the SMC (Frey et al., 2009; Schnurr-Pütz et al., 2006; Shestak and Busse, 2005) by reducing pore space, restricting access to biological components, and impeding microbial activity (Balser and Firestone, 2005). The observed increase in anaerobic bacteria and the CYC stress ratio in the mechanical control during the first spring may indicate that some level of compaction occurred; however, no other microbial metrics or soil microclimate variables differed between the mechanical control and unharvested control treatments. Other research at this site has found no differences in soil respiration (Stoffel et al., 2010) or vegetation (Burton, 2011) between mechanical control and unharvested control treatments.

4.2. Early response – importance of labile C and soil moisture

The responses of AMF and single-celled soil bacteria to group selection harvests are directly related to belowground labile C allocation and soil moisture (Fig. 1). Initially post-harvest, symbiotic

(Smith and Read, 1997) and rhizodeposition (Farrar et al., 2003) C inputs decrease, resulting in consistently low relative abundance of AMF during the first 4 years. This is compounded by the high soil moisture in gaps, even during periods with lower than average precipitation, which leads to the large differences in AMF abundance between gaps and controls during the first and second summer. Increased soil moisture in gaps also corresponds with a greater abundance of anaerobic bacteria during the first 2 years.

Gram positive soil bacteria are often considered k-strategists (MacArthur and Wilson, 1967); they are generally highly efficient microorganisms that use complex C sources, are successful in nutrient limited situations (Ringelberg et al., 2008), and have thick cell walls making them resistant to moisture stress (Schimel et al., 2007). Gram negative soil bacteria are more aligned with an r-strategy (MacArthur and Wilson, 1967) with rapid growth from use of labile C sources, lower nutrient or moisture stress tolerance, and poor competitive success with other organisms (Deslippe et al., 2012; Fierer et al., 2007; Ringelberg et al., 2008; Treonis et al., 2004). Therefore, the initial increase in GmP bacterial abundance could be due to their ability to use existing (non-labile) sources of soil C and maintain cell osmotic pressures under moisture stress, making them more competitive than GmN bacteria in harvests during the first year post-harvest. When GmN bacteria become stressed, they convert monounsaturated (16:1ω7c; 18:1ω7c) to cyclopropyl fatty acids (17:0cyc; 19:0cyc) (Guckert
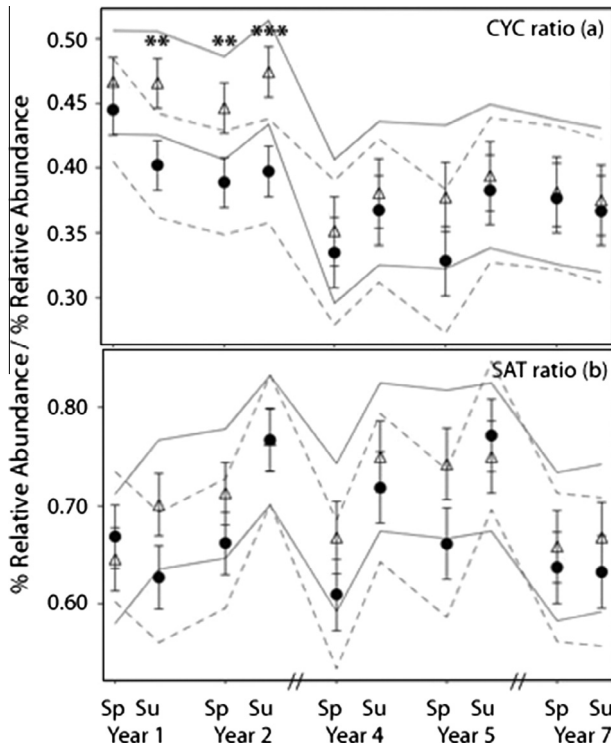
Fig. 5. Gap (open triangles) and control (closed circles) treatment means for CYC and SAT microbial stress ratios in spring (Sp) and summer (Su) of each sampling year. Sample sizes are $n = 4$ for years 1 and 2, and $n = 5$ for years 4, 5, 7. Means are graphed with standard error bars and 95% confidence intervals (gap = solid lines, and control = dotted lines). Asterisks represent pairwise significance levels (*$p < 0.1$; **$p < 0.05$; ***$p < 0.01$; ****$p < 0.001$).
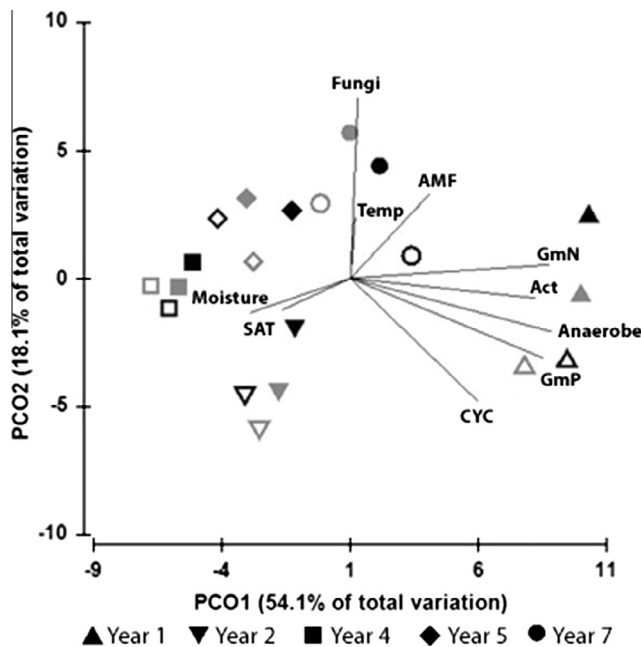
Fig. 6. PCO analysis of gap and control treatment group centroids through time. Sample sizes are $n = 8$ for years 1 and 2 (4 large, 4 medium subplots), and $n = 5$ (large subplots) for years 4, 5, 7. Pearson correlation vector overlays ($r > 0.2$) of microbial guilds, soil temperature and moisture are presented. Black symbols = control; grey symbols = harvested gap. Closed symbols = spring sampling period; open symbols = summer sampling period.

et al., 1986), and unsaturated to saturated fatty acids (Kieft et al., 1997), resulting in increased CYC and SAT ratios. While we do not observe a strong SAT ratio response to harvesting, the CYC ratio was significantly higher in harvested gaps than controls during the early sampling periods, indicating initial GmN bacterial stress followed by recovery as trees regrew and labile C sources were reintroduced to the soil.

4.3. Mid-term response – importance of recalcitrant C

The medium and large group selection harvests caused significant reductions in the fine root C pool for at least 3 years post-treatment due to root death (Forrester et al., 2013). This influx of belowground plant litter material is considered recalcitrant due to lignins, tannins, cutins, and other chemically recalcitrant components, which can only be decomposed by specific soil microorganisms that produce strong oxidative agents (Rasse et al., 2005). Neither recalcitrant C decomposer group responded to increased inputs during the first 2 years post-harvest; however, in the later years, actinomycetes became less abundant while saprotrophic fungi were more abundant in harvested gaps, significantly so during the spring of year 7. This response has been previously identified (Brant et al., 2006), and may be due to species competition and succession dynamics. Actinomycetes can have an antagonistic effect on fungal activity and growth (Wohl and McArthur, 2001), while fungi can inhibit actinomycetes by increasing substrate acidification (de Boer et al., 2005). Following harvest, fine roots initially decay rapidly due to a high proportion of soluble carbohydrates and ease of accessibility to decomposers. Larger roots decay slower because more time is required for fungal colonization (Fahey et al., 1988). The increase in fungal abundance in the latter years following harvest could be due to the decomposition lag time of large roots and stumps within canopy gaps. Additionally, the reduction of actinomycetes in later years may be caused by the prior decomposition of readily accessible, fine-root biomass, and competition/inhibition due to a larger fungal community.

4.4. Harvest versus annual and seasonal effects

Although our primary objective was focused on the response of the SMC to harvest treatment, the variation in SMC composition observed in our dataset was overwhelmingly influenced by sampling year and seasonal differences. The higher significance of seasonal variation over treatment effects is important to note; if only one season were sampled our interpretation of treatment effects may be misleading. For instance, you would expect to see reduced AMF abundance in harvested gaps compared to unharvested controls in this ecosystem (Fig. 1); however, if you were to only look during the spring, your chances of observing this difference would be reduced due to higher soil moisture. Other studies have also reported large seasonal shifts in SMC structure due to variations in temperature, moisture, and resource availability (Bossio et al., 1998; Kaiser et al., 2010; Moore-Kucera and Dick, 2008). These differences due to sampling season may be an additional cause of the wide range of variability observed in the response of the SMC to forest harvesting (Table 1). The magnitude of yearly and seasonal variability may result in a SMC that is more resilient to current and future climate change due to increased adaptability to extreme temperature or moisture (Gutknecht et al., 2012).

AMF were more abundant during the warm, dry summer sampling periods; a relationship we can clearly observe during the summer of year 1, when extremely dry controls coincide with a highly significant increase in AMF abundance. Low moisture in the control, as opposed to the harvested gap, was due to below average seasonal precipitation and above average temperatures (Fig. 2), as well as less water uptake by plants in gaps, leading to
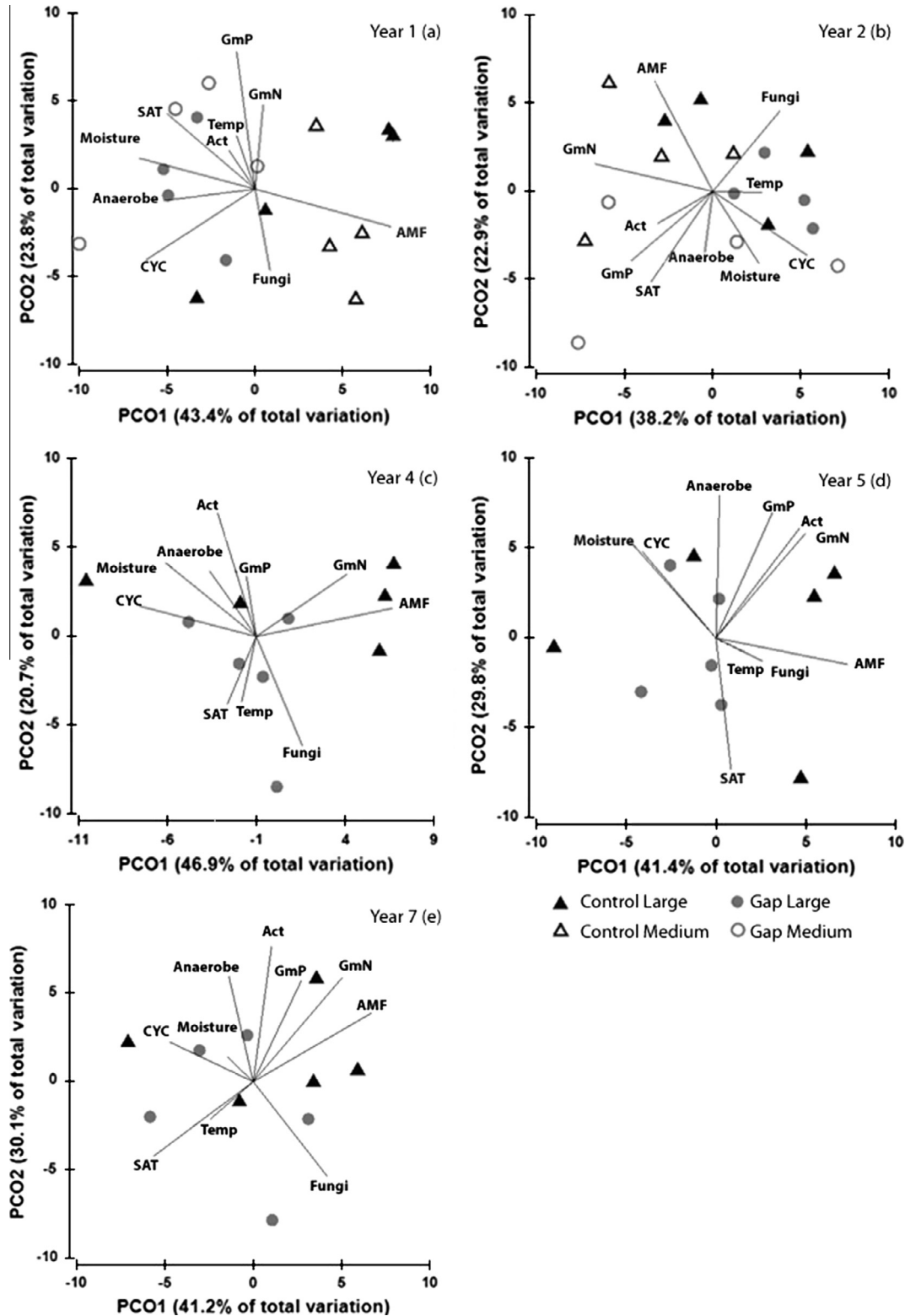
Fig. 7. PCO graphs of gap and control SMC in summer of years sampled. Pearson correlation vector overlays (>0.2) of microbial guilds, soil temperature and moisture are presented.

greater soil moisture. Microbes with a filamentous growth form are better able to resist moisture stress because they can translocate water from other parts of the soil matrix, making them more competitive in dry conditions (Schimel et al., 2007). In contrast, GmN bacteria were generally more abundant during the cool,

wet spring and either remained constant or decreased in abundance during the summer. There are two potential causes of this observed pattern. First, low GmN abundance during the summer may be due to moisture (Schimel et al., 2007) or temperature (Deslippe et al., 2012; Zogg et al., 1997) stress, resulting in

generally higher stress ratios during the hot, dry summers (Kieft et al., 1997). Second, because GmN bacteria are a responsive initial sink for labile C sources in microbial rhizosphere communities (Bird et al., 2011), the increase in labile C during vegetation leaf-out may initially increase GmN abundance during the spring.

5. Conclusions

This research provides evidence that group selection harvests producing canopy gaps 200–380 m^2 in size cause immediate shifts in SMC composition. After 2–4 years these differences disappear and the SMC begins to resemble unharvested controls. Our results suggest that microbial groups that use recent C sources or respond to changes in microclimate are responsible for the initial changes in the SMC composition (AMF, GmP, GmN), while groups that use complex C sources, such as woody debris or roots, do not respond to gap selection harvest for up to 4 years (fungi, actinomycetes). In addition to resource availability, competition between microbial groups may play a role in structuring the SMC. These harvesting effects may be specific to the fertile, second growth forest type we studied, which had its original primary forest logged 100 years ago, and do not address repeated or short-rotation forestry. Finally, this study emphasizes the importance of seasonal variation in structuring the SMC. This high temporal variability reinforces the need for repeated sampling over multiple seasons. Analysis of one sampling period may be insufficient to fully characterize management effects when the temporal response fluctuates both seasonally and annually.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foreco.2014.12.012.

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