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Response of the soil microbial community and soil nutrient bioavailability to biomass harvesting and reserve tree retention in northern Minnesota aspen-dominated forests

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ABSTRACT

Intensive forest biomass harvesting, or the removal of harvesting slash (woody debris from tree branches and tops) for use as biofuel, has the potential to negatively affect the soil microbial community (SMC) due to loss of carbon and nutrient inputs from the slash, alteration of the soil microclimate, and increased nutrient leaching. These effects could result in lowered forest productivity and threaten the long-term sustainability of forest management. Retaining organic material post-harvest, including greater amounts of harvesting slash and live trees, within harvested areas may ameliorate some negative effects of biomass harvesting on soil processes. We evaluated the effects of biomass harvests with reserve tree and slash retention on the SMC and soil nutrient bioavailability (assessed using plant-root simulator probes) in trembling aspen (*Populus tremuloides* Michx.) forests in northern Minnesota during the spring and summer, 1–3 years after harvest. Variable biomass removal levels tested include complete removal (whole tree harvest of boles and branches), complete slash retention (bole only harvest), and 20% slash retention (amount suggested by regional biomass harvesting guidelines). Compared to the unharvested control, biomass harvests had no effect on the multivariate SMC composition or microbial biomass, but did result in a 1–4% increase in arbuscular mycorrhizal fungal abundance and reduced bacterial stress two and three years after harvest. Additionally, biomass harvesting increased NH_4 bioavailability during year one, and reduced NO_3 bioavailability during year two when compared to unharvested controls. Among the three biomass harvests with differing levels of slash removal there were few differences in overall SMC composition, microbial biomass, and soil nutrients; however, the abundance of arbuscular mycorrhizal fungi, gram positive and actinomycete bacteria were significantly higher in harvested treatments with more slash retained. These results are specific to single rotation biomass harvesting in aspen stands due to the unique relationships between plants and their associated SMCs, and may not be directly applicable to forest biomass harvesting of other commercial forest tree species, or multiple rotations.

1. Introduction

Concerns about the effects of increasing atmospheric CO_2 and rising fossil fuel costs prompted legislation in the US (The Energy Policy Act of 2005; The Energy Independence and Security Act of 2007) that encouraged the removal of previously non-merchantable woody material, including tree branches and tops, as energy feed stocks (Janowiak and Webster, 2010). Although the energy derived from this type of biomass harvesting could potentially result in a net reduction of CO_2 emissions to the atmosphere (Schlamadinger and Marland, 1996), biomass harvesting can also have negative effects on the forest ecosystem, particularly on long-term sustainability of forest soil productivity (Grigal, 2000; Thiffault et al., 2011).

The soil microbial community (SMC) is directly related to ecosystem productivity (Zak et al., 2003) through its role in the decomposition of carbon (C) sources and organic matter, which generate bioavailable nutrients that can be utilized by plants

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(McGuire and Treseder, 2010). Almost all forest management activities affect forest soils; however, the magnitude of this effect ranges over a broad gradient and depends on direct and indirect harvesting effects on soil properties such as bulk density, soil nutrients and C, the habitat and substrate for the SMC, and micrometeorological conditions affecting SMC activity and abundance (Grigal, 2000). Forest harvesting can alter the composition of the SMC directly (Hannam et al., 2006; Hynes and Germida, 2012; Lewandowski et al., 2015; Moore-Kucera and Dick, 2008; Siira-Pietikäinen et al., 2001), influencing nutrient cycling and soil fertility (Hynes and Germida, 2013).

Whole-tree harvesting (WTH), where tree boles, tops and branches are removed intact, can remove twice the amount of nutrients as a bole only harvest (BOH) (Alban et al., 1978; Perala and Alban, 1982), potentially causing long-term soil productivity declines (Paré et al., 2002). WTH also can lead to a net loss of soil C from the A horizon (Johnson and Curtis, 2001). This may be partly due to the use of heavier machinery in a WTH that open up more travel lanes, resulting in accelerated C removals and greater post-harvest C emissions (Mika and Keeton, 2012). In addition, low amounts of residual slash following WTH can reduce C inputs to the soil as opposed to BOH, where higher slash loads that supply recalcitrant C may result in microbial nitrogen (N) immobilization and reduce nutrient leaching after harvest (Covington, 1981). WTH can also lead to increases in soil temperature (Devine and Harrington, 2007; Slesak, 2013) due to greater irradiance of the soil surface, which can affect the SMC and nutrient cycling. In an effort to address the negative effects of WTH, many states have enacted management guidelines that seek to retain appropriate amounts of slash (Evans et al., 2010 North East State Foresters Association, 2012); however, little research has quantified the minimum amount of slash retention necessary to maintain soil fertility and ecosystem function.

Another strategy to mitigate the potential long-term negative effects of WTH on soil properties is the retention of live trees within the harvested area (Janowiak and Webster, 2010). Retained trees can provide refugia for above and belowground biota, and may be important in maintaining C and nutrient cycling dynamics (Franklin et al., 2007; Gustafsson et al., 2012) by retaining continuity of the structural, functional, and compositional attributes of a stand from pre- to post-harvest (Gustafsson et al., 2012). Retained trees can provide a source of mycorrhizal inoculum (Amaranthus and Perry, 1994), and increase percent mycorrhizal colonization (Outerbridge and Trofymow, 2009). Additionally, retained trees help to maintain a soil microclimate, and physical and chemical properties that are more similar to conditions in unharvested areas than the surrounding harvest (Barg and Edmonds, 1999), and supply C through symbiotic interactions, litterfall and rhizodeposition. Also, retained trees may decrease macronutrient leaching through continued plant uptake (Brais et al., 2004; Lindo and Visser, 2003; Prescott, 1997).

In this research, we experimentally tested the response of the SMC (evaluated using phospholipid fatty acid (PLFA) analysis) to variable slash retention levels (all slash retained, i.e., BOH, 20% slash retention, and all slash removed, i.e. WTH) and live tree reserve patches (aggregates of trees approximately 0.1 ha in size) for three years following biomass harvests. In addition we evaluated the response of soil nutrient availability for the first two years following biomass harvests (both whole tree and bole only harvests). To accomplish this, we used a replicated, operational (large-scale) harvesting experiment in mature trembling aspen (*Populus tremuloides* Michx.) dominated forests in northern Minnesota. Aspen is a fast-growing, nutrient-use intensive tree species whose commercial importance and wide range throughout the upper Great Lakes region, USA (Alban et al., 1991, 1978) make it an ideal species to evaluate impacts of biomass harvesting and mitigation options. To our knowledge, this is the first study to evaluate the response of soil microorganisms to a level of slash retention that is similar to the biomass harvesting guidelines that are currently used in multiple states (including Minnesota, Michigan, Missouri, Pennsylvania, and Wisconsin) in the USA.

Our first objective evaluates the effects of biomass harvesting in general by comparing the three harvested treatments with the unharvested control. We expected that harvested areas would have higher arbuscular mycorrhizal fungal abundance compared to the

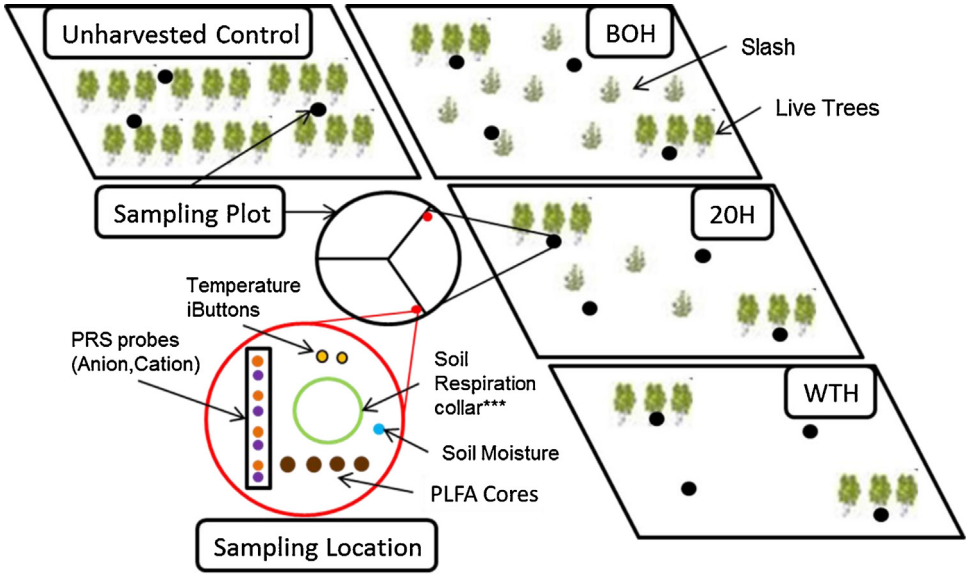

Fig. 1. Diagram of site-level sampling layout. At each site (4 total), we sampled from 4 biomass harvesting stands: one unharvested control, and three harvests with varying levels of slash retention (BOH, 20H, WTH) and tree reserve patches within each stand. Within control stands, we sampled from 3 sampling plots; within the harvested stands, we sampled from 2 sampling plots within the harvested area and two within the reserve patches, one in each patch. At each sampling plot, data were collected from two sampling locations of 1 m in diameter, located 11.3 m from plot center at 30° and 150° azimuth.
(BOH = Bole-only harvest, all harvesting slash retained; 20H = 20% of harvesting slash retained; WTH = Whole-tree harvest, no harvesting slash retained).
***Soil respiration collar data from years 1–3 after harvest previously published in Kurth et al. (2014).

control (Hannam et al., 2006; Visser et al., 1998), and that harvesting would result in reduced microbial biomass (Hassett and Zak, 2005), though SMC composition would remain relatively unchanged (Hannam et al., 2006; Hassett and Zak, 2005). We also expected that harvested areas would have greater nutrient availability than the unharvested control due to reduced plant uptake. Our second objective compares the three biomass harvests with different levels of slash retention to each other. We hypothesize that retaining greater amounts of biomass post-harvest will mitigate some of the negative effects of a WTH, resulting in reduced nutrient availability, increased microbial biomass and microbial communities that are more similar to the unharvested control.

2. Methods

2.1. Site description

This research was performed at four replicated, experimental sites in St. Louis County, Minnesota, USA (47°0′N, −92°24′W; 47°15′N, −92°19′W; 48°1′N, −92°59′W; 48°9′N, −92°59′W). Soils at all four sites are glacial till-derived loams, ranging from stony and sandy to silty in texture (see Slesak 2013; Table 1). Climate is cold-temperate continental, with cool, short summers and long, cold winters. The frost free growing season is typically 60–100 days. Average air temperature ranged from −16 °C in January to 26 °C in July and mean annual precipitation ranged from 660 to 710 mm, primarily occurring between May and October (Klockow et al., 2013). Prior to treatment, stands were dominated by 55- to 68-year old aspen originating by spouting of lateral roots following harvest. Other tree species present included *Betula papyrifera* Marshall, *Acer rubrum* L., *Fraxinus nigra* Marshall., *Abies balsamea* (L.) Mill., *Picea mariana* (Mill.) Britton, and *Picea glauca* (Moench) Voss, with occasional *Thuja occidentalis* L. and *Pinus strobus* L. Further site descriptions can be found in Klockow et al. (2013), Slesak (2013), and Kurth et al. (2014).

2.2. Experimental design

The four sites served as replicate blocks in a randomized complete block design. Each site contained four treatments applied at a stand-scale (4.1 ha): (1) all slash retained (BOH) with live tree reserve patches, (2) WTH with 20% slash retained (20H) and live tree reserve patches, (3) no slash retained (WTH) with live tree reserve patches, and (4) an unharvested control. In the 20H treatment, 20% of the original slash in the stand was retained by either retaining the slash of 1 in 5 harvested trees directly on site, or by piling the slash on an adjacent landing and returning 20% of the slash back to the stand. Within each harvested stand, 5% of the area was maintained in two roughly square or rectangular reserve patches of live trees (0.1 ha each). In the biomass harvests (BOH, 20H, WTH), data were collected from two sampling plots within the harvested matrix, and 2 sampling plots within the reserve patches (one in each patch) in each stand. In the unharvested controls, data were collected from 3 sampling plots (Fig. 1). Stands were clearcut harvested under frozen ground conditions during February 2010 using ground-based mechanized equipment (a feller-buncher and grapple skidder).

2.3. Soil microbial community

Soil samples for PLFA analysis were collected during the spring (late May–early June) and summer (mid-August) of 2010, 2011, and 2012; one, two, and three growing seasons after harvest, respectively. Within each sampling plot, soil was collected at two sampling locations that were 11.3 m from plot center at 30° and 150° azimuths. At each sampling location, four soil cores were taken to a depth of 15 cm (generally including A and E soil horizons) and composited into one sample using a 2.36 cm diameter push probe (Hoffer sampler, JBK, Beavercreek, OH) (Fig. 1). Forest floor litter (leaves and sticks) was cleared from the soil surface prior to core collection. Soil samples were stored at −20 °C prior to being lyophilized (Freezemobil 12, Virtis of Gardiner, NY). Roots and stones were removed from dried samples, and samples were ground in preparation for microbial lipid extraction.

Phospholipids were extracted from 3.5 g of lyophilized soil using a two-phase, aqueous-organic, phosphate buffer-methanol-chloroform extraction, developed from a modified PLFA and fatty-acid methyl ester (FAME) method (Balser and Firestone, 2005). Each sample was extracted twice, and then the organic phase was isolated and dried down in a RapidVap (LabConco, Kansas City, MO), saponified, subjected to alkaline methanolysis, and isolated in hexane. The resulting FAMEs from the extracted phospholipids were analyzed using a Hewlett-Packard 6890 Gas Chromatograph with a flame ionization detector configured and maintained for lipid analysis according to the recommendations of MIDI (MIDI Inc., Newark DE). Peaks were identified by comparing retention times with known standards using the MIDI Sherlock microbial identification system (MIS) software (MIDI Inc., Newark DE). To quantify lipid amounts, peak areas were first multiplied by a response factor (Rfact), which corrects for differences in detector response across the range of chain-lengths (Christie, 1989), and is derived from the MIDI calibration standards. Once peak areas are Rfact corrected, lipids can be quantified by comparison with Rfact corrected external standards (9:0, 19:0) of known concentration. Raw lipid data were processed using an open source licensed Microsoft Access® Database (Devin Wixon, 2013, Lipid GC Process) to obtain absolute (μmol lipid/g soil) and relative (mol%) abundances. Absolute abundances for all lipids were summed to represent total microbial biomass (Balser and Firestone, 2005; Hill et al., 1993; White et al., 1979; Zelles et al., 1992). Lipids with a relative abundance (averaged over all samples) of less than 0.5 mol % were removed, leaving 29 PLFAs in the dataset. With the exception of microbial biomass, relative abundance lipid data was used for all analyses. Fatty acid nomenclature is as described elsewhere (Frostegård et al., 1996; Zelles, 1997; Aanderud et al., 2008).

Specific indicator lipids were classified into microbial guilds, including: arbuscular mycorrhizal fungi (AMF) (16:1ω5); Fungi (18:2ω6,9); Gram Positive bacteria (GmP) (14:0iso, 15:0anteiso, 15:0iso, 16:0iso, 17:0anteiso, 17:0iso); Gram Negative bacteria (GmN) (16:1ω7, 18:1ω7, 19:0 cyclo, 17:0 cyclo); Actinomycetes (Act.) (16:0 10 methyl) (Bossio et al., 1998; Frostegård et al., 1996, 1993; Kieft et al., 1997; Olsson, 1999; Vestal and White, 1989; Wilkinson, 1988; Zelles, 1999; Zelles et al., 1992). Additionally, we analyzed the ratio of cyclopropyl to monoenoic fatty acids (17:0cyc, 19:0cyc/16:1ω7c, 18:1ω7c) as the CYC stress ratio; increases in this ratio indicates nutritional and/or anaerobic stress in the SMC (Guckert et al., 1986; Kieft et al., 1997, 1994).

2.4. Soil nutrient availability

Within BOH with live tree reserve patches, WTH with live tree reserve patches, and unharvested control treatments, Plant Root Simulator (PRS™) probes were used to monitor *in situ* bioavailable soil macronutrients during the first and second growing seasons after harvest (Western Ag. Innovations, Saskatoon, SK) at the same sampling locations as PLFA soil core collection (Fig. 1). During the first two growing seasons, four anion and four cation probes were installed vertically into the mineral soil concurrently with the first microbe collection during the spring, and then removed during the

second microbe collection during the summer for a total bioavailable nutrient collection period of 12 weeks (Western Ag. Innovation, PRS^{TM} probe Operations Manual). Following collection, the PRS probes were thoroughly washed with distilled water to remove residual soil particles, combined into one sample per location, and shipped to Western Ag. Innovations for macronutrient extraction (NO_3, NH_4, Ca, Mg, K, P).

2.5. Data analysis

We used a weighted-average approach to scale the microbial and nutrient data collected in harvested and reserve patch plots to a stand-level based on the respective areas of each (Fig. 1). The plot-level microbial PLFAs, microbial biomass, guilds, stress ratio, and soil nutrient means were multiplied by the proportion of harvested area (0.951) or the reserve patch area (0.049), then summed for a stand-level estimate. To characterize the response of the multivariate soil microbial community, we performed a permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001), in PRIMER version 6 (Clarke and Gorley, 2015) with the PERMANOVA+ add-on package (Anderson et al., 2008) using a Bray–Curtis resemblance measure on untransformed relative abundance PLFA data. PERMANOVAs were run using 9999 random permutations, Type III sum of squares, and permutation of residuals under a reduced model. Mixed model analysis of variance was performed in SAS version 9.3 (SAS, 2008; System for Windows, SAS Institute Inc., Cary, NC, USA) to analyze the response of microbial biomass, guilds, the stress ratio, and soil nutrients. A mixed-effects design was used for both PERMANOVA and ANOVA analyses to test the fixed effects of biomass harvesting stands and the unharvested control, year, and season, with stands (and their interactions) nested within sites as a random effect to account for spatial variability and the repeated nature of the sampling. Soil nutrients were analyzed similarly, except season was not a factor in the model.

In Appendix A, we also include a mixed-effects split-plot ANOVA to compare the response of the SMC and soil nutrients in reserve patches to the harvested matrix, with treatment (BOH, 20H, WTH) at the whole-plot level and sub-treatment (harvest, reserve) at the sub-plot level. While not part of the essential results, we report it as part of the overall design. Stands were nested within sites as a random effect, with year, and season as fixed effects except for soil nutrients where season was not a factor in the model (Appendix A: Table A.1).

3. Results

3.1. Stand-level response of the SMC and soil nutrients to biomass harvesting treatments

The multivariate composition of the SMC did not differ due to biomass harvesting treatments based on the PERMANOVA analysis (Table 1). Total microbial biomass was also not significantly affected by treatments during any time period sampled; however, AMF, GmP and actinomycete bacteria, and the CYC stress ratio all exhibited significant variation due to biomass harvesting treatments, or treatment interactions with yearly or seasonal factors (Table 1). AMF were more abundant in every biomass harvest compared to unharvested controls, starting in the second summer after harvest and continuing into the third year (both spring and summer). Among the three harvest treatments, AMF tended to be lowest in WTH; however, this difference was only significant between 20H and WTH during the second summer (Fig. 2a). GmP and actinomycete bacterial abundance were significantly higher when more slash was retained (BOH and 20H) compared to WTH treatments (Fig. 2b and c). Nutritional and/or anaerobic stress as indicated by the CYC stress ratio varied among slash levels depending on both the season and the year (Table 1). By the third year, the ratio was significantly lower than the unharvested treatments in all slash levels, largely driven by summer conditions (Fig. 2d and e). In general, year, season, and the interaction of year by season explained a significant portion of the variability in the microbial response when comparing biomass harvest and unharvested control treatments (Table 1).

Nitrogen availability differed among harvests and unharvested control treatments by year since harvest (treatment × year interaction; NH_4 $p = 0.09$, NO_3 $p = 0.02$). Soil NH_4 responded immediately to harvesting treatments, with significantly higher bioavailability ($p = 0.01$) in BOH and WTH compared to unharvested controls during year 1 (Table 2). NO_3 responded to treatments in the second year, with significantly lower ($p < 0.01$) availability in BOH and WTH relative to unharvested controls (Table 2). Soil Ca, Mg, K, and P bioavailability did not differ due to treatment effects (Table 2). NH_4 bioavailability was significantly higher during year 1 ($p < 0.05$), while NO_3, Ca, and P bioavailability was significantly higher during year 2 (Table 2).

4. Discussion

4.1. General stand-level effects of biomass harvests with reserve tree retention

Based on the PERMANOVA analysis, we did not find significant differences in the multivariate SMC composition among biomass harvesting treatments 1–3 years following harvest; a result corroborated by previous studies in harvested aspen stands 4.5–5.5 (Hannam et al., 2006) and 8–10 (Hassett and Zak, 2005) years following forest harvest. Contrary to what we expected, we also found no significant differences in microbial biomass among experimental biomass harvesting treatments. Conclusions from previous research in aspen forests have been mixed. In a study of variable slash retention, microbial biomass was significantly lower than the unharvested control only following complete slash removal (WTH) (Hassett and Zak, 2005). In studies of live tree reserves, Hannam et al. (2006) found no effect of 20% and 50% live tree reserve treatments on microbial biomass compared to the

Table 1
P-values ($p < 0.1$) from the analysis of the effects of biomass harvesting stands and controls (treatment) over 3 sampling years (year) and 2 seasons each year (season) on the multivariate SMC (PERMANOVA), microbial biomass (μmol lipid/g soil), guilds (% relative abundance), and the bacterial stress ratio (%/%) (ANOVA).

	df	SMC Composition	Biomass	AMF	GmP	GmN	Fungi	Act	CYC
Treatment	3	–	–	0.09	0.02	–	–	0.03	–
Year	2	0.0001	0.06	0.0001	0.02	0.0001	0.003	0.0008	–
Treatment × Year	6	–	–	0.03	–	–	–	–	0.08
Season	1	0.0001	–	0.0001	–	–	–	–	0.0001
Treatment × Season	3	–	–	0.0003	–	–	–	–	0.05
Year × Season	2	0.0001	0.009	0.0001	0.004	–	0.0001	–	0.009
Treatment × Year × Season	6	–	–	0.02	–	–	–	–	–

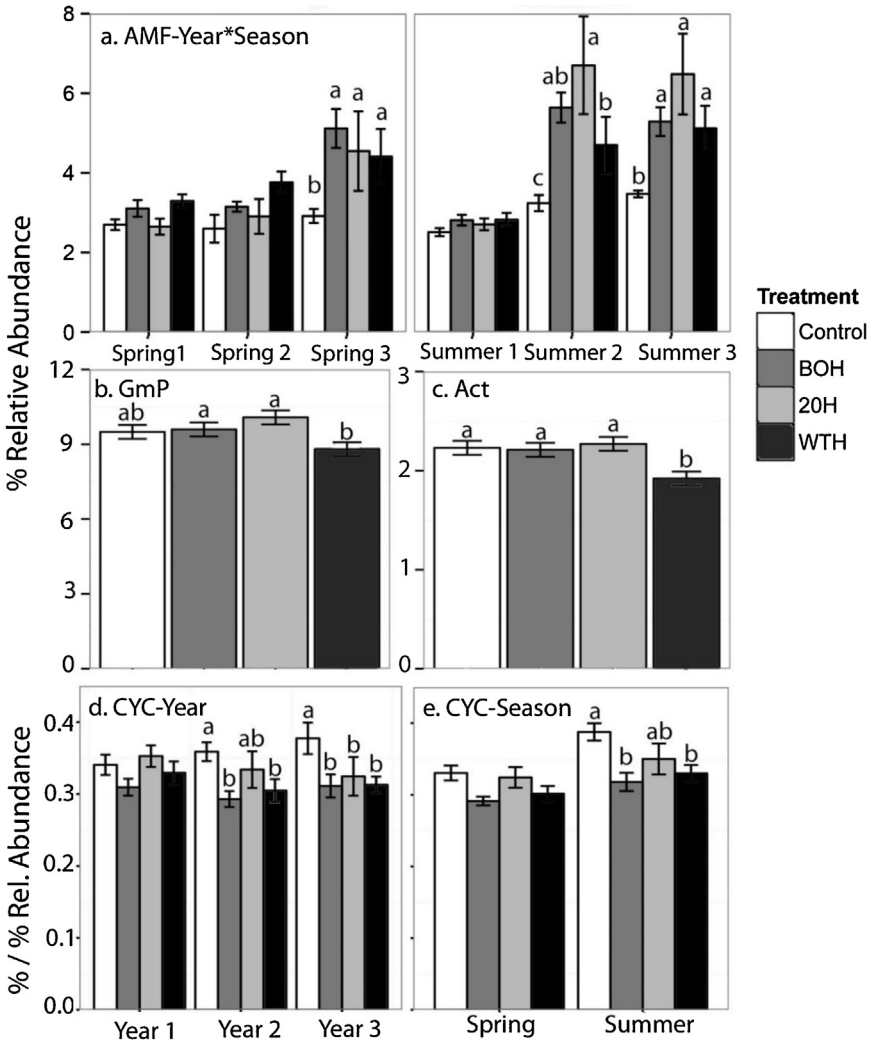

Fig. 2. Significant effects ($p \leq 0.05$) of biomass harvesting treatments on (a) AMF (treatment × year × season), (b) GmP and (c) actinomycete bacteria (treatment effect averaged over all sampling periods), and d and e. the CYC stress ratio (treatment × year; treatment × season). Letters above bars indicate significant differences between controls and biomass harvests ($p \leq 0.05$), while the absence of letters indicates no significant differences. Error bars represent standard error of the mean.

Table 2
Average soil nutrient availability (μg/10 cm^2/12 weeks) in biomass harvesting (BOH and WTH) stands and controls during the summer of years 1 and 2 post-harvest. Standard deviations are indicated in parentheses following the mean.

Treatment	Year	NO_3	NH_4	Ca	Mg	K	P
Control	1	3.2 (1.0)	6.9 (1.3)	1885 (350)	394 (65)	43.6 (26)	6.7 (5.5)
BOH	1	4.9 (4.4)	14.9 (5.7)	1994 (30)	348 (58)	28.7 (14)	11.2 (3.5)
WTH	1	3.9 (3.9)	15.0 (6.4)	1896 (131)	332 (38)	22.3 (13)	10.4 (5.8)
Control	2	15.6 (3.8)	6.4 (1.4)	1893 (263)	377 (62)	38.1 (20)	9.6 (4.9)
BOH	2	8.7 (3.0)	7.4 (1.6)	2198 (91)	361 (69)	32.2 (15)	14.7 (4.7)
WTH	2	11.0 (2.4)	5.9 (1.1)	2127 (128)	368 (28)	32.0 (19)	13.2 (7.7)

unharvested control. Lindo and Visser (2003) found that microbial biomass was lower in uncut live tree reserve areas and adjacent harvested corridors than the unharvested control, but were not different from a clearcut harvest. Research on the effects of harvesting with various slash retention levels in conifer stands more consistently found a reduction in microbial biomass following harvest (Busse et al., 2006; Chatterjee et al., 2008; Hynes and Germida, 2013; Moore-Kucera and Dick, 2008; Siira-Pietikäinen et al., 2001). While we cannot definitively explain why aspen harvesting seems to have less of a consistent effect on microbial biomass than harvesting of other tree species, a possible explanation is the quick resprouting of aspen suckers following harvest, resulting in an extremely dense, 1–2 m tall aspen monoculture by year 3 post-harvest (personal observation). Aspen are unique from many commercial tree species in that they are clonal and regenerate quickly via vegetative root suckering following aboveground harvest (Alban et al., 1991; Frey et al., 2003), generating a highly interconnected forest stand (DesRochers and Lieffers, 2001; Shepperd, 1993). The quick regrowth of aspen after harvesting, coupled with an

interconnected root system that can redistribute photosynthetically derived C and maintain rhizodeposition could explain the low effect on overall SMC composition and microbial biomass.

Based on previous research (Hannam et al., 2006; Visser et al., 1998), we expected that biomass harvesting would cause an increase in AMF abundance; a result we observed during years 2 and 3 after harvest. This increase is most likely related to the 70% increase in herbaceous plant cover following harvest (Curzon, 2014), while during year 1, high soil moisture in harvests (Kurth et al., 2014) most likely inhibited AMF growth (Mentzer et al., 2006; Visser et al., 1998). We also observed a reduced CYC stress ratio in harvests, which indicates a sufficient labile C supply and low bacterial stress (Guckert et al., 1986; Kieft et al., 1997). We speculate that this may be due to aspen being a fast-growing, clonal tree species that quickly re-sprouts after cutting (Alban et al., 1991; Frey et al., 2003) and resumes C cycling (Lee et al., 2002), including belowground labile C inputs through rhizodeposition. Compared to unharvested controls, biomass harvests had high NH_4 bioavailability during year 1, and low NO_3 bioavailability during year 2. High NH_4 initially may have been caused by reduced plant uptake within the harvest (Marks and Bormann, 1972), but we are unable to adequately explain the reduction in NO_3 during year 2.

Compared to the harvested matrix, reserve patches had reduced plant available NH_4, NO_3 and P (Appendix A), possibly due to continued plant uptake. Reserve patches also had high AMF abundance and reduced bacterial stress compared to the surrounding harvest; a result most likely due to the maintenance of a more favorable soil microclimate in reserve patches. However, this effect was ephemeral, and by years 2 and 3 reserve tree patches and the harvested matrix become more similar to each other and less similar to the unharvested control (Appendix A: Table A.1, Fig. A.1).

4.2. Effects of slash retention in stand-level biomass harvests

Contrary to what we expected, soil nutrient bioavailability was not different between WTH and BOH treatments. However, we did see a few effects of slash retention level on the SMC. These differences in the SMC among slash retention levels were difficult to explain because they were not linearly related to the amount of slash retained. A factor potentially contributing to these trends is the generally higher soil temperatures in WTH (Kurth et al., 2014) due to the lower amount of residual slash left on site (Klockow et al., 2013), which may have lowered AMF, GmP, and actinomycete abundance. It is also possible that the harvesting disturbance associated with 20H is different than other treatments, resulting in a unique SMC response. For instance, greater soil disturbance may have occurred from multiple entries during harvest (i.e., back-hauling slash from landings onto the site) (Grigal, 2000), or slash piling may have resulted in extreme variation in soil temperature and moisture across a harvest unit (Moroni et al., 2009) and affected the SMC in 20H differently than either BOH or WTH treatments. Additionally, we cannot rule out that the lack of major differences among the slash retention levels may be due to the actual levels of slash retention in the field being more similar to each other than the prescribed levels (Klockow et al., 2013), which may have equalized harvesting treatments to some extent. Finally, because this was a deciduous forest harvested in the winter, nutrient rich leaves were not removed with the harvest, which may have reduced the initial sensitivity to biomass removal treatments.

5. Conclusion

We found that variable levels of intensive biomass harvesting with reserve trees in aspen dominated forests had minimal effects on overall multivariate SMC composition or total microbial biomass, but did influence AMF abundance, bacterial stress, and N bioavailability two and three years after harvest compared to the unharvested control. The amount of slash removed during the harvest did not cause a significant shift in the multivariate SMC composition, total microbial biomass, or soil nutrients within the first three years following harvest, but did alter AMF, GmP, and actinomycete relative abundances and bacterial stress.

These responses represent the immediate effects of intensive forest biomass harvesting and live tree reserves on the SMC and soil nutrient bioavailability in aspen stands. To more fully understand the implications for long-term forest sustainability, further sampling is needed on a longer time scale to allow slash to decompose and the stand to regrow. While this study is important in furthering our understanding of the ecological effects of biomass harvesting and alternative management techniques, it is important to note that these results are only applicable to forest biomass harvests in aspen due to the above–belowground feedbacks that shape the unique relationships between plants and soil microbes in these and other communities. Additional research on the ecological effects of intensive biomass harvesting and mitigation techniques in a range of forest types is needed to make broader conclusions regarding the effects of these practices on forest ecosystem services and long-term forest sustainability.

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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.apsoil.2015.11.001.

References

Aanderud, Z.T., Shuldman, M.I., Drenovsky, R.E., Richards, J.H., 2008. Shrub-interspace dynamics alter relationships between microbial community composition and belowground ecosystem characteristics. Soil Biol. Biochem. 40, 2206–2216. doi:http://dx.doi.org/10.1016/j.soilbio.2008.04.008.

Alban, D., Perala, D., Jurgensen, M., Ostry, M., Probst, J., 1991. Aspen ecosystem properties in the upper Great Lakes. USDA For. Serv. St. Paul MN NC-300, 47.

Alban, D.H., Perala, D.A., Schlaegel, B.E., 1978. Biomass and nutrient distribution in aspen, pine, and spruce stands on the same soil type in Minnesota. Can. J. For. Res. 8, 290–299. doi:http://dx.doi.org/10.1139/x78-044.
Amaranthus, M., Perry, D., 1994. The functioning of ectomycorrhizal fungi in the field: linkages in space and time. Plant Soil 159, 133–140.
Anderson, M., Gorley, R., Clarke, R., 2008. Permanova+ for PRIMER: Guide to Software and Statistical Methods. PRIMER-E Ltd., Plymouth, UK.
Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecol. 26, 32–46.
Balser, T.C., Firestone, M.K., 2005. Linking microbial community composition and soil processes in a California annual grassland and mixed-conifer forest. Biogeochemistry 73, 395–415. doi:http://dx.doi.org/10.1007/sl0533-004-0372-y.
Barg, A.K., Edmonds, R.L., 1999. Influence of partial cutting on site microclimate, soil nitrogen dynamics, and microbial biomass in Douglas-fir stands in western Washington. Can. J. For. Res. 29, 705–713. doi:http://dx.doi.org/10.1139/cjfr-29-6-705.
Bossio, D.A., Scow, K.M., Gunapala, N., Graham, K.J., 1998. Determinants of soil microbial communities: effects of agricultural management, season, and soil type on phospholipid fatty acid profiles. Microb. Ecol. 36, 1–12.
Brais, S., Harvey, B.D., Bergeron, Y., Messier, C., Greene, D., Belleau, A., Paré, D., 2004. Testing forest ecosystem management in boreal mixedwoods of northwestern Quebec: initial response of aspen stands to different levels of harvesting. Can. J. For. Res. 34, 431–446. doi:http://dx.doi.org/10.1139/x03-144.
Busse, M.D., Beattie, S.E., Powers, R.F., Sanchez, F.G., Tiarks, A.E., 2006. Microbial community responses in forest mineral soil to compaction, organic matter removal, and vegetation control. Can. J. For. Res. 36, 577–588. doi:http://dx.doi.org/10.1139/x05-294.
Chatterjee, A., Vance, G.F., Pendall, E., Stahl, P.D., 2008. Timber harvesting alters soil carbon mineralization and microbial community structure in coniferous forests. Soil Biol. Biochem. 40, 1901–1907. doi:http://dx.doi.org/10.1016/j.soilbio.2008.03.018.
Christie, W.W., 1989. Gas Chromatography and Lipids, 3rd ed. The Oily Press Ltd., Bridgewater, Somerset.
Clarke, K., Gorley, R., 2015. PRIMER v7: User Manual/Tutorial. PRIMER-E Ltd., Plymouth, UK.
Covington, W.W., 1981. Changes in forest floor organic matter and nutrient content following clear cutting in northern hardwoods. Ecology 62, 41–48.
Curzon, M., 2014. Productivity, Recovery, Diversity, and Function of Aspen-dominated Forests Vary in Response to Biomass Harvest Severity. Retrieved from the University of Minnesota Digital Conservancy, http://hdl.handle.net/11299/167041.
DesRochers, A., Lieffers, V.J., 2001. Root biomass of regenerating aspen (*Populus tremuloides*) stands of different densities in Alberta. Can. J. For. Res. 31, 1012–1018. doi:http://dx.doi.org/10.1139/cjfr-31-6-1012.
Devine, W.D., Harrington, C.A., 2007. Influence of harvest residues and vegetation on microsite soil and air temperatures in a young conifer plantation. Agric. For. Meteorol. 145, 125–138. doi:http://dx.doi.org/10.1016/j.agrformet.2007.04.009.
Evans, A., Perschel, R., Kittler, B., 2010. Revised Assessment of Biomass Harvesting and Retention Guidelines. The Forest Guild, Santa Fe, NM.
Franklin J.F., Mitchell R.J., Palik B.J., 2007. Natural disturbance and stand development principles for ecological forestry. Gen. Tech. Rep. NRS-19., USDA For. Serv. Gen. Tech. Rep. NRS-19. Newtown Square, PA.
Frey, B.R., Lieffers, V.J., Landhäusser, S.M., Comeau, P.G., Greenway, K.J., 2003. An analysis of sucker regeneration of trembling aspen. Can. J. For. Res. 33, 1169–1179. doi:http://dx.doi.org/10.1139/x03-053.
Frostegård, Å., Tunlid, A., Bååth, E., 1993. Phospholipid fatty acid composition biomass, and activity of microbial communities from two soil types experimentally exposed to different heavy metals. Appl. Environ. Microbiol. 59, 3605–3617.
Frostegård, Å., Tunlid, Å., Bååth, E., 1996. Changes in microbial community structure during long-term incubation in two soils experimentally contaminated with metals. Soil Biol. Biochem. 28, 55–63.
Grigal, D.F., 2000. Effects of extensive forest management on soil productivity. For. Ecol. Manag. 138, 167–185. doi:http://dx.doi.org/10.1016/s0378-1127(00)00395-9.
Guckert, J.B., Hood, M.A., White, D.C., 1986. Phospholipid ester-linked fatty acid profile changes during nutrient deprivation of Vibrio cholerae: increases in the trans/cis ratio and proportions of cyclopropyl fatty acids. Appl. Environ. Microbiol. 52, 794–801.
Gustafsson, L., Baker, S., Bauhus, J., Beese, W., Brodie, A., Kouki, J., Lindenmayer, D.B., Lõhmus, A., Pastur, G.M., Messier, C., Neyland, M., Palik, B., Sverdrup-thygeson, A., Volney, W.J.A., Wayne, A., Franklin, J.F., 2012. Retention forestry to maintain multifunctional forests: a world perspective. Bioscience 62, 633–645. doi:http://dx.doi.org/10.1525/bio.2012.62.7.6.
Hannam, K., Quideau, S., Kishchuk, B., 2006. Forest floor microbial communities in relation to stand composition and timber harvesting in northern Alberta. Soil Biol. Biochem. 38, 2565–2575. doi:http://dx.doi.org/10.1016/j.soilbio.2006.03.015.
Hassett, J., Zak, D., 2005. Aspen harvest intensity decreases microbial biomass, extracellular enzyme activity, and soil nitrogen cycling. Soil Sci. Soc. Am. J. 69, 227–235.
Hill, T., McPherson, E., Harris, J., Birch, P., 1993. Microbial biomass estimated by phospholipid phosphate in soils with diverse microbial communities. Soil Biol. Biochem. 25, 1779–1786. doi:http://dx.doi.org/10.1016/0038-0717(93)90183-c.
Hynes, H.M., Germida, J.J., 2012. A chronsequential approach to investigating microbial community shifts following clearcutting in boreal plain forest soils. Can. J. For. Res. 42, 2078–2089. doi:http://dx.doi.org/10.1139/cjfr-2012-0038.
Hynes, H.M., Germida, J.J., 2013. Impact of clear cutting on soil microbial communities and bioavailable nutrients in the LFH and Ae horizons of boreal plain forest soils. For. Ecol. Manage. 306, 88–95. doi:http://dx.doi.org/10.1016/j.foreco.2013.06.006.
Janowiak, M., Webster, C., 2010. Promoting ecological sustainability in woody biomass harvesting. J. For. 108, 16–23.
Johnson, D., Curtis, P., 2001. Effects of forest management on soil C and N storage: meta analysis. For. Ecol. Manag. 140, 227–238. doi:http://dx.doi.org/10.1016/s0378-1127(00)00282-6.
Kieft, T.L., Ringelberg, D.B., White, D.C., 1994. Changes in ester-linked phospholipid fatty acid profiles of subsurface bacteria during starvation and desiccation in a porous medium. Appl. Environ. Microbiol. 60, 3292–3299.
Kieft, T.L., Wilch, E., O'Connor, K., Ringelberg, D.B., White, D.C., 1997. Survival and phospholipid fatty acid profiles of surface and subsurface bacteria in natural sediment microcosms. Appl. Environ. Microbiol. 63, 1531–1542.
Klockow, P.A., D'Amato, A.W., Bradford, J.B., 2013. Impacts of post-harvest slash and live-tree retention on biomass and nutrient stocks in Populus tremuloides Michx. -dominated forests, northern Minnesota, USA. For. Ecol. Manag. 291, 278–288. doi:http://dx.doi.org/10.1016/j.foreco.2012.11.001.
Kurth, V.J., Bradford, J.B., Slesak, R.A., D'Amato, A.W., 2014. Initial soil respiration response to biomass harvesting and green-tree retention in aspen-dominated forests of the Great Lakes region. For. Ecol. Manag. 328, 342–352. doi:http://dx.doi.org/10.1016/j.foreco.2014.05.052.
Lee, J., Morrison, I.K., Leblanc, J.-D., Dumas, M.T., Cameron, D.A., 2002. Carbon sequestration in trees and regrowth vegetation as affected by clearcut and partial cut harvesting in a second-growth boreal mixedwood. For. Ecol. Manag. 169, 83–101. doi:http://dx.doi.org/10.1016/s0378-1127(02)00300-6.
Lewandowski, T.E., Forrester, J.A., Mladenoff, D.J., Stoffel, J.L., Gower, S.T., D'Amato, A.W., Balser, T.C., 2015. Soil microbial community response and recovery following group selection harvest: Temporal patterns from an experimental harvest in a US northern hardwood forest. For. Ecol. Manag. 340, 82–94. doi:http://dx.doi.org/10.1016/j.foreco.2014.12.012.
Lindo, Z., Visser, S., 2003. Microbial biomass, nitrogen and phosphorus mineralization, and mesofauna in boreal conifer and deciduous forest floors following partial and clear-cut harvesting. Can. J. For. Res. 33, 1610–1620. doi:http://dx.doi.org/10.1139/x03-080.
Marks, P.L., Bormann, F.H., 1972. Revegetation following forest cutting: mechanisms for return to steady-state nutrient cycling. Science 176, 914–915. doi:http://dx.doi.org/10.1126/science.176.4037.914.
McGuire, K.L., Treseder, K.K., 2010. Microbial communities and their relevance for ecosystem models: decomposition as a case study. Soil Biol. Biochem. 42, 529–535. doi:http://dx.doi.org/10.1016/j.soilbio.2009.11.016.
Mentzer, J.L., Goodman, R.M., Balser, T.C., 2006. Microbial response over time to hydrologic and fertilization treatments in a simulated wet prairie. Plant Soil 284, 85–100. doi:http://dx.doi.org/10.1007/s11104-006-0032-1.
Mika, A.M., Keeton, W.S., 2012. Factors contributing to carbon fluxes from bioenergy harvests in the U.S. Northeast an analysis using field data. GCB Bioenergy doi:http://dx.doi.org/10.1111/j.1757-1707.2012.01183.x.
Moore-Kucera, J., Dick, R.P., 2008. PLFA profiling of microbial community structure and seasonal shifts in soils of a douglas-fir chronosequence. Microb. Ecol. 55, 500–511. doi:http://dx.doi.org/10.1007/s00248-007-9295-l.
Moroni, M.T., Carter, P.Q., Ryan, D.A.J., 2009. Harvesting and slash piling affects soil respiration, soil temperature, and soil moisture regimes in Newfoundland boreal forests. Can. J. Soil Sci. 89, 343–355.
North East State Foresters Association, 2012. A Review of Biomass Harvesting Best Management Practices Guidelines. North East State Foresters Association.
Olsson, P.A., 1999. Signature fatty acids provide tools for determination of the distribution and interactions of mycorrhizal fungi in soil. FEMS Microbiol. Ecol. doi:http://dx.doi.org/10.1111/j1574-6941.1999.
Outerbridge, R.A., Trofymow, J.A.T., 2009. Forest management and maintenance of ectomycorrhizae: a case study of green tree retention in south-coastal British Columbia. BC J. Ecosyst. Manag. 10, 59–80.
Paré, D., Rochon, P., Brais, S., 2002. Assessing the geochemical balance of managed boreal forests. Ecol. Indic. 1, 293–311. doi:http://dx.doi.org/10.1016/s1470-160x(02)00026-2.
Perala, D.A., Alban, D.H., 1982. Biomass, nutrient distribution and litterfall in Populus, Pinus and Picea stands on two different soils in Minnesota. Plant Soil 64, 177–192.
Prescott, C.E., 1997. Effects of clearcutting and alternative silvicultural systems on rates of decomposition and nitrogen mineralization in a coastal montane coniferous forest. For. Ecol. Manag. 95, 253–260. doi:http://dx.doi.org/10.1016/s0378-1127(97)00027-3.
Schlamadinger, B., Marland, G., 1996. The role of forest and bioenergy strategies in the global carbon cycle. Biomass Bioenergy 10, 275–300.
Shepperd, W.D., 1993. Initial growth, development, and clonal dynamics of regenerated aspen in the Rocky Mountains. USDA For. Serv. Res. Pap. RM-312.
Siira-Pietikäinen, A., Pietikäinen, J., Fritze, H., Haimi, J., 2001. Short-term responses of soil decomposer communities to forest management: clear felling versus alternative forest harvesting methods. Can. J. For. Res. 31, 88–99. doi:http://dx.doi.org/10.1139/cjfr-31-1-88.
Slesak, R.A., 2013. Soil temperature following logging-debris manipulation and aspen regrowth in Minnesota: implications for sampling depth and alteration of

soil processes. Soil Sci. Soc. Am. J. 77, 1818. doi:http://dx.doi.org/10.2136/sssaj2013.01.0022.
Thiffault, E., Hannam, K., Paré, D., 2011. Effects of forest biomass harvesting on soil productivity in boreal and temperate forestséa review. Environ. Rev. 19, 278–309. doi:http://dx.doi.org/10.1139/A11-009.
Vestal, J., White, D., 1989. Lipid analysis in microbial ecology. Bioscience 39, 535–541.
Visser, S., Maynard, D., Danielson, R., 1998. Response of ecto- and arbuscular mycorrhizal fungi to clear-cutting and the application of chipped aspen wood in a mixedwood site in Alberta, Canada. Appl. Soil Ecol. 7, 257–269.
White, D.C., Davis, W.M., Nickels, J.S., King, J.D., Bobbie, R.J., 1979. Determination of the sedimentary microbial biomass by extractible lipid phosphate. Oecologia 40, 51–62. doi:http://dx.doi.org/10.1007/bf00388810.
Wilkinson, S.G., 1988. Gram negative bacteria. In: Ratledge, C., Wilkinson, S.G. (Eds.), Microbial Lipids. Academic Press, London, pp. 299–488.
Zak, D.R., Holmes, W.E., White, D.C., Peacock, A.D., Tilman, D., 2003. Plant diversity, soil microbial communities, and ecosystem function: are there any links? Ecology 84, 2042–2050.
Zelles, L., 1997. Phospholipid fatty acid profiles in selected members of soil micobial communities. Chemosphere 35, 275–294.
Zelles, L., 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. Biol. Fertil. Soils 29, 111–129. doi:http://dx.doi.org/10.1007/s003740050533.
Zelles, L., Bai, Q.Y., Beck, T., Beese, F., 1992. Signature fatty-acids in phospholipids and lipopolysaccharides as indicators of microbial biomass and community structure in agricultural soils. Soil Biol. Biochem 24, 317–323.